MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXXVII MARCH–APRIL, 1945 NO. 2

OBSERVATIONS ON THE GENUS CATENARIA [1]

JOHN N. COUCH

(WITH 78 FIGURES)

INTRODUCTION

In soil from the Gulf States collected in January 1942, a fungus was found growing within the threads of *Allomyces anomalus* unlike any parasite previously seen. Many of the threads of *Allomyces* were filled from base to tip with ovoid or spherical, colorless or pale brown *Olpidium*-like bodies (FIG. 2). Subcultures of the infected *Allomyces* were made, but before observations were carried out, the cultures had dried. Upon the addition of water, the pale brown bodies of the parasite germinated, much as in *Blastocladiella cystogena*. Observation showed that the resting sporangia, as well as the zoosporangia, were not formed singly as in *Olpidium*, but were connected one to another by narrow isthmuses exactly as in *Catenaria Anguillulae*. Here apparently was a fungus connecting the *Blastocladiales* with *Catenaria* which might throw some light on that still inadequately known genus. It is the purpose of this paper to describe the new parasite on *Allomyces* and to report some heretofore unknown facts in the life history of *Catenaria Anguillulae* which will help to clear up the confusion about that genus.

[1] Address of the President, 1943, Mycological Society of America, Cleveland, Ohio, September 14, 1944.

[MYCOLOGIA for January–February (37: 1–162) was issued February 3, 1945.]

OBSERVATIONS ON CATENARIA ALLOMYCIS

The new fungus, which I will call *Catenaria Allomycis,* was purified by adding the zoospores of the parasite sucked up in a capillary pipet to water cultures of the *Allomyces* growing on hemp seed. The water cultures of the host were descended from a pure culture on agar F_{13}. All attempts to culture the parasite with or without its host on agar have resulted in failure. Some of the agars tried were 0.5 per cent plain agar, 1 per cent agar plus a trace of peptone, F_{13} agar (first used by Foust for the successful growth of *Allomyces* and its parasite *Rozella Allomycis*) and various strengths of liver agar with and without various sugars (for formulas see below under *C. Anguillulae*). For a fungal parasite to be transmitted through its host on solid media it must be capable of producing schizonts which spread through the host. *R. Allomycis* does this, while *C. Allomycis* does not. All attempts to culture *C. Allomycis* on cooked plant material, as several kinds of grass leaves, onion leaves and roots, and cooked animals as nematodes and fluke eggs failed.

The parasite first found on *Allomyces anomalus* has been transferred to all other species of *Allomyces.* It grows well on both the gametophytic and sporophytic generations of *A. arbusculus* and *A. javanicus,* having the same appearance as on the original host. However, it attacks *A. javanicus* so greedily that by the second generation, the host is destroyed before it can form resting bodies. It is doubtful if the parasite could survive in nature on this plant. On *A. moniliformis* it is a less vigorous parasite than on *A. anomalus,* and produces many resting bodies singly in the zoosporangia of the host (FIG. 12). The parasite grows well on *Blastocladiella simplex* Matthews, but not at all on *B. cystogena, B. laevisperma,* or *B. asperosperma.* It failed to grow on *Blastocladia parva, Catenaria Anguillulae, Achlya caroliniana,* and *Saprolegnia parasitica.*

Resting bodies of the parasite retain their vitality dried for a year and perhaps much longer. Stock material for study and experimental purposes is prepared simply by allowing infected cultures of *Allomyces* to dry up in a Petri dish. If charcoal water is added, the resting sporangia of both host and parasite begin to germinate in a few hours (10–24) and new cultures are made by adding

pieces of boiled hemp seed. A simple procedure has been to add water and hemp seed to dried cultures late in the afternoon and by the following morning the resting bodies would show all stages of germination.

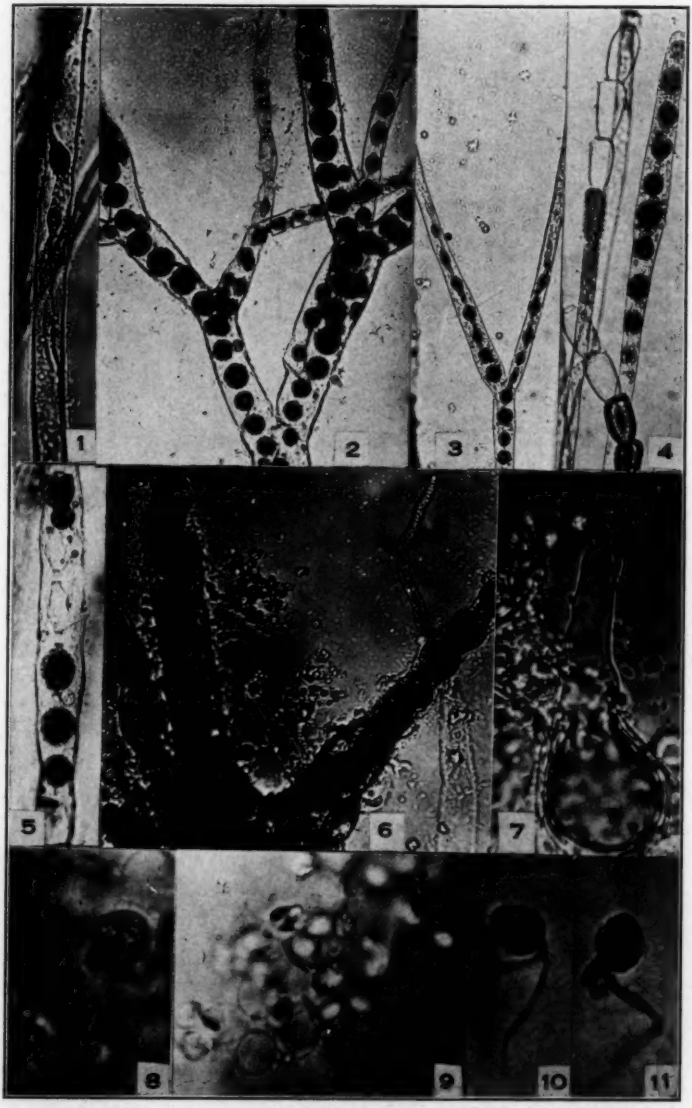
Experiments have shown that the resting bodies in any given culture are not ready for germination all at the same time. Six cultures dried for 22 days showed 5–10 per cent germination of the resting bodies when water was added. These six were dried again, and after two months showed a total resting body germination of 15–20 per cent. Immediately after the observations were made the six cultures were dried again, this time for five months. Upon the addition of water and after a lapse of 36 hours, all six contained vast numbers of germinating resting bodies.

In germinating, the resting body absorbs water, the fat bodies become more or less uniform in size and are more evenly distributed in the cytoplasm. The swelling of the contents causes the outer wall of the resting body to crack on one side into several irregular lobes and through this opening a broad tube pushes (FIG. 35). This penetrates the host wall through a large opening and may extend out to a length of 50–several hundred microns (FIGS. 6, 7, 37–40). The development of the resting body zoospores is similar to that of the regular zoospores, except that here the fat bodies are more abundant and are collected in a sphere some time before the spores emerge (FIGS. 36–40). A similar condition has been shown in *Blastocladiella simplex* for the zoospores by Matthews (1937) and in *Allomyces arbusculus* for the gametes by Hatch (1935). When mature, the tip of the tube gelatinizes and the resting body zoospores emerge, the first ones usually collecting in a ball. As in *Blastocladiella cystogena,* the resting body zoospores are furnished with cilia, one to each spore, and are capable of a little sluggish swimming, but never progress far from the tip before encysting (FIG. 37). As a rule, the resting body zoospores encyst at the tip in an irregular compact mass (FIGS. 38–40). Each spore contains a nucleus, nuclear cap and many fat bodies (FIG. 37).

After about two hours, the contents of the cysts divide into four gametes, which emerge through a short papilla (FIGS. 6, 8, 9, 38–41). They usually remain for several minutes at the tip of the papilla, their cilia projecting straight out and occasionally lashing

in a futile fashion (FIG. 41). Sometimes one may break from the cluster and swim or crawl away, or rarely the entire cluster of four may swim awkwardly away like a colony of *Pascheriella*. Usually some of the gametes remain stationary at the exit pore while others creep about over the entire mass. When two compatible gametes come together side by side with their cilia pointed in the same direction, they fuse so rapidly that the actual process is easily overlooked. The two membranes become one, the nuclear caps fuse and then the nuclei unite. The shiny globules do not unite but all collect on one side of the zygote and toward the posterior end. The two cilia do not unite but, as in *Blastocladiella cystogena*, become so closely appressed one to another as to appear as one and act as one in swimming (FIGS. 10, 11, 41, 42). At times when the zygote pauses for a moment, the two cilia can be seen under the dark field to separate for a moment and lash independently. Stained preparations also show two distinct cilia (FIGS. 10, 42).

There is much variation in the behavior of the gametes. A few special cases will be described. Three quiet gametes were selected for observation. Two or three minutes later one became active and began crawling about over the other two, and then suddenly broke loose and swam away. The other two remained in contact with very gentle ciliary motion for about five minutes, and then began fusing. As this progressed, the immature zygote became active and swam away before fusion was entirely complete. This observation is of interest because it shows that two compatible gametes may lie in contact some time before fusing. Other observations indicated that sometimes gametes may behave as males and females. This was the case with a gamete found sitting on a cyst. It was inactive except for an occasional lash of its flagellum. Soon another gamete, a male (?) swam up, and began creeping over the surface of the female. About two minutes later a second male came on the scene and immediately both began fusing with the female. However, the first gamete to arrive had the better start and as the zygote took form, it succeeded in jerking loose from the second male. The above description of gamete formation and fusion is strikingly similar to the behavior in *Blastocladiella cystogena* (Couch & Whiffen, **1942**).



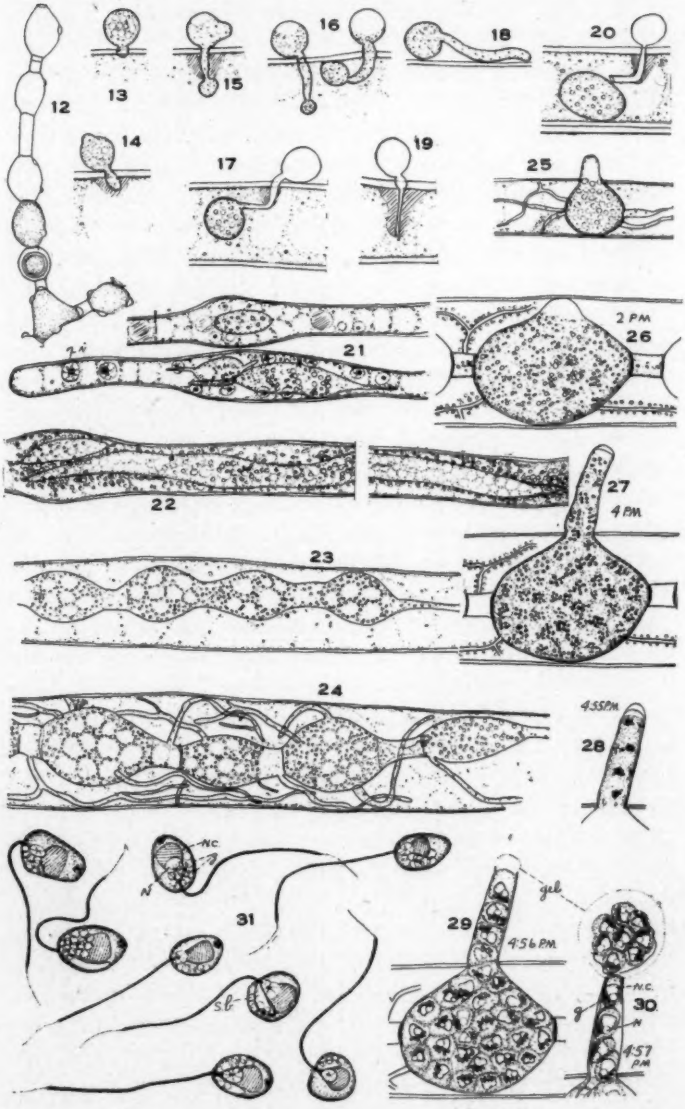
FIGS. 1–11. *Catenaria Allomycis.*

The zygote is a vigorous swimmer. Eventually it comes to rest on the surface of the host, loses its cilia, rounds up, encysts, and sends a fine penetration tube into the host thread (FIGS. 13, 44). As the tube grows, the host deposits considerable callus material around the tube but only seldom is its growth checked (FIGS. 17, 19, 20). If no host is present, the zygote may form a germ tube in water, exactly like the one that penetrates the host. This tube grows to a maximum length equal to about twice the diameter of the zygote, and then changes to a zoosporangium to form two zoospores.

The infecting body is round at first but soon elongates (FIG. 20), becoming somewhat spindle-shaped (FIG. 21). The ends continue to grow, extending lengthwise in the host thread until a long, irregular, tubular structure, usually unbranched, is formed (FIG. 22). It is very difficult to make out the limits of the parasitic thallus at this stage, for it is surrounded by the fat bodies and protoplasm of the host. It appears that the immature thallus is devoid of rhizoids. When it has attained mature length, swellings begin to form at more or less regular intervals (FIG. 23). When these are about mature in size, septa are laid down separating the swellings from one another. At first only one septum is laid down between two swellings, and this is always at the end of the isthmus and never in the middle (FIG. 24). A few minutes later, after most of the protoplasm has moved from the isthmus into the swollen part, another septum is formed separating the isthmus from the swelling. The enlarged parts become the zoosporangia or resting sporangia and the narrow parts the isthmuses (FIGS. 24, 26).

The rhizoids are thick, stubby structures, and apparently are not developed until after the thallus becomes septate. They are usually surrounded by the granular or fatty material of the host (FIGS. 26, 27). In figure 24 the rhizoids are exceptionally well developed. On the thalli shown in figures 21–23 no rhizoids could be detected. The young thallus apparently absorbs its food over its entire surface. The rhizoids in this species are perhaps vestigial structures.

The mature thallus of the parasite typically consists of a single, usually unbranched catenulate hypha within the host. However, a single host hypha may contain two or even several moniliform



FIGS. 12–31. *Catenaria Allomycis.*

parasite thalli (FIG. 32) or the thalli may be clustered in a swollen portion of the host thread (FIG. 33).

The first infections of a young culture by the zygotes of *Catenaria Allomycis* produce parasitic thalli consisting entirely of zoosporangia. The zoospores formed in these sporangia give rise to thalli that may be part zoosporangia and part resting bodies and the later formed zoospores give rise to thalli consisting entirely of resting bodies. It seems that resting body formation is brought on by the exhaustion of the host thallus and a reduction of the food, as Barrett (1912) suggested in *Olpidiopsis*. Evidence for this conclusion is that it is possible to keep zoosporangial thalli going constantly by adding fresh hemp seed daily so that new young *Allomyces* will always be present.

Just before sporangial formation, the protoplasm moves from the isthmuses into the swellings and these become separated from the isthmuses by cross walls (FIG. 24). The swollen parts become the zoosporangia or resting sporangia. The protoplasm at this stage contains numerous vacuoles and fat bodies of variable size. A sporangium mature in size had the appearance shown in figure 26. The emergence tube had just begun to grow out. Two hours later (FIG. 27), the tube was formed and the fat bodies, now all of about the same size, were arranged in clusters of about eight. Each cluster of fat bodies indicated the center of a spore origin. Vacuoles at this stage were doubtless present, but very indistinct. About an hour later (FIG. 28), the protoplasm showed a very homogeneous condition, except for the fat bodies. During this phase a new moon-shaped body appeared in the cytoplasm. This quickly enlarged and formed the nuclear cap, surrounding half of the nucleus which meanwhile had become distinct. As the nuclear caps formed, the spores became very distinct and were polygonal from pressure (FIG. 29). While this was happening (and taking only a few seconds), the tip gelatinized and suddenly the end spore followed by others pushed out into a spherical gelatinous envelope formed from the gelatinizing tip (FIGS. 29, 30). For a few seconds the spores in the sphere remained quiet, but soon became active, broke through the surrounding gelatine, and swam away with a single posterior cilium. The remaining spores with the pressure now released became very active, swimming about in the sporangium and push-

ing up into and through the tube to the outside, to swim away immediately.

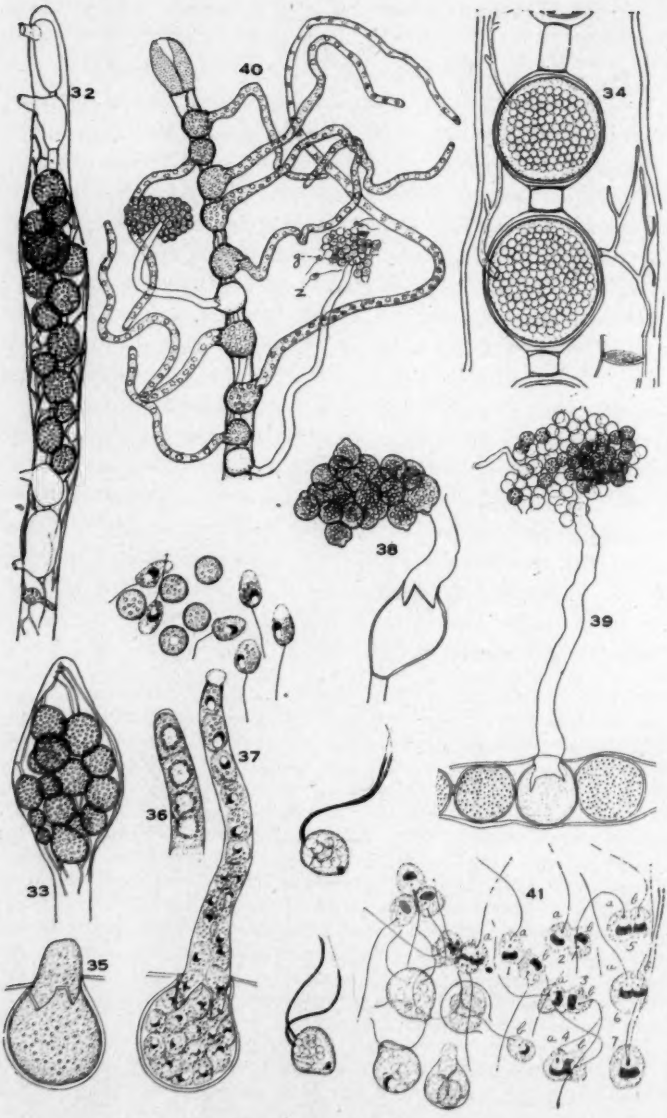
Zoospores were killed in a drop of water on a slide by exposure in a closed chamber to the fumes of 1 per cent osmic acid and stained immediately by stirring into the drop a small amount of freshly prepared aqueous solution of crystal violet. Such preparations may be studied immediately and show the whiplash cilium, the nucleus and nuclear cap, the side body apparently attached at one end to the base of the cilium, and the several fat bodies (FIG. 31). Gametes and zygotes (FIG. 42) have been stained in the same way.

Resting bodies appear as the host becomes exhausted. Their early development is as in the zoosporangia. Indeed in maturing cultures it is difficult to determine if a swelling is going to form a zoosporangium or resting body, until the papilla begins to form or the resting body wall begins to thicken. In a few instances, resting bodies have formed in zoosporangia which had developed papilla. When the resting body is formed, it shrinks slightly from the zoosporangial wall and then forms about itself a thick outer wall and a thin inner membrane (FIG. 34). The germination of the resting bodies has already been described.

Catenaria Allomycis, sp. nov.

In hyphis *Allomycis* et *Blastocladiellae simplicis* parasitica. Hyphae ramosae vel simplices, catenulatae cum rhizoideis paucis brevibus; hyphae 300–600 μ longitudine, ex zoosporangiis vel sporangiis perdurantibus catenulatim conjunctis per angustos isthmos compositae. Sporangia globosa vel oblongo-elliptica, 15–30 $\times$ 30–55 μ; zoosporae ovoideae, 5–6.3 $\times$ 6.3–7 μ. Cellulae perdurantes fulvosae vel leniter rubellae, globosae vel subglobosae, pariete levi vel minute punctato; siccatae germinantes per rimas irregulares parietis externi crassi; tubulo exeunte 50 ad aliquot 100 μ longitudine. Zoosporae ex cellulis perdurantibus uniflagellatae tempore dimissionis, prope tubulum exeuntem in irregulari cumulo in sporas immotas mox se convertentes. Sporae immotae sphericae, 5.4–6.4 μ diametro, post temporis spatium breve germinates et quattuor uniflagellatas cellulas sexuales formantes quae emergunt et per paria copulant. Cellulae sexuales 3 $\times$ 4.4–4.7 μ. Zygosporae biflagellatae, 3.5 $\times$ 7.6 μ, germinantes et hospitem penetrantes formantes thallum qui zoosporangia gignit.

Thallus parasitic in the threads of *Allomyces* and *Blastocladiella;* consisting when mature of a simple or branched catenulate hypha with a few stubby rhizoids; usually 300–600 μ long, consisting of 6–15 zoosporangia or resting bodies or both, sometimes much



FIGS. 32–41. *Catenaria Allomycis.*

longer and at times much shorter, being composed of only one zoosporangium or resting body; the swellings in the thallus connected by one-celled very rarely 2-celled short isthmuses, 5–9.2 × 7–16 μ. Septations incompletely formed, bumpy, pitted, or ridged. The first developed thalli forming zoosporangia, the later ones zoosporangia and resting bodies, and the last thalli only resting bodies. Zoosporangial development much as in *Allomyces* or *Blastocladiella simplex*. Zoospores with numerous fat bodies or lipoid granules, a conspicuous nuclear cap and a side body (visible only when stained), posteriorly uniflagellate. First zoospores emerging in a gelatinous envelope, the later ones emerging and swimming away upon reaching the exit; swimming smoothly as in *Blastocladiella*. Zoosporangia globose, subglobose, or pyriform to long elliptic or oblong elliptic, usually oval or ovoid; 15–30 × 30–55 μ; emergence tube 5–6 μ thick × 10–100 μ long. Zoospores oval, 5–6.3 × 6.3–7 μ, cilium about 17 μ long and tail piece 6.3 μ long. Resting bodies pale brown to pinkish; usually globose or subglobose, ovoid, elliptic, pyriform, rarely somewhat irregular in shape. On *Allomyces anomalus* 16–51 μ when globose, 30–41 × 34–42 when subglobose; on *A. javanicus* considerably larger, up to 63 μ thick when globose; wall of resting body 1–3 μ thick, smooth or very minutely rough; the whole spore surrounded by a thin-walled case which it almost completely fills. Resting body germinating by irregular cracking of the outer thick wall and the emergence of a germ tube which varies from 50–several hundred μ in length. Resting body zoospores uniciliate when discharged and capable of a little feeble movement, encysting almost immediately near the emergence pore in an irregular mass. Cysts spherical, 5.4–6.4 μ thick. After a rest period of about 2 hours germinating to form four uniciliate gametes which emerge through a pore to fuse in pairs, gametes 3 × 4.4–4.7 μ. Zygotes biciliate, 3.5–7.6 μ. Germinating and penetrating the host to form a zoosporangial thallus.

Found only once on *Allomyces anomalus* in soil collected by J. N. and Philip Couch from Texas, January 2, 1942.

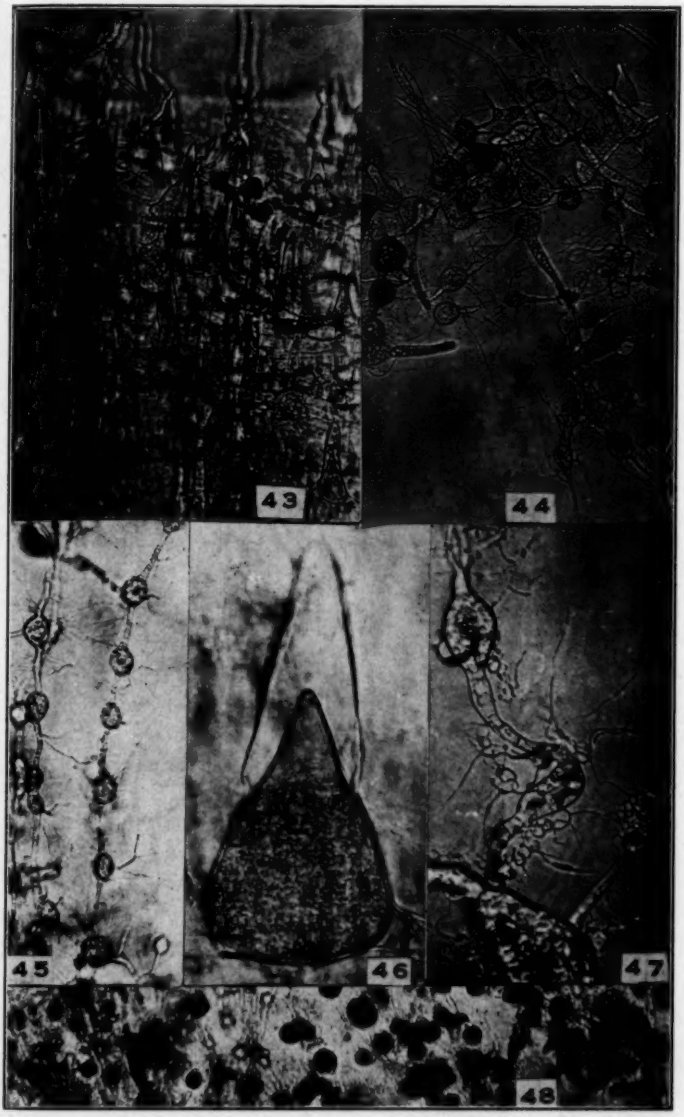
In its life cycle the above fungus shows a striking parallel to that of *Allomyces cystogenus* while in thallus structure it resembles *Catenaria Anguillulae*.

OBSERVATIONS ON CATENARIA ANGUILLULAE SOROKINE

According to the figures of Buckley and Clapham (**1929**) the resting body membrane of *Catenaria Anguillulae* cracks in germinating in the same distinctive manner as it does in the fungus

from Texas and in *Blastocladiella cystogena*. It seemed possible that *Catenaria Anguillulae*, which has heretofore been classified in the Chytridiales, might be more closely related to the Blastocladiales. It was imperative, therefore, that *C. Anguillulae* be studied again and compared with the fungus parasitic on *Allomyces anomalus*.

An intensive search was started for *Catenaria Anguillulae*. Sorokine and others had reported it on nematodes in Europe and Karling (**1934**) in this country had reported it on the roots of cooked grass, onions, several cooked algae and on the sterilized eggs of rotifers, infusoria and insects from New York City. Several collections of nematodes both free living and the species causing root knot of tomato were made and kept in wet soil from cow pastures but none of the nematodes were found parasitized by *Catenaria*. Boiled onion roots, which Karling (**1934**) found to be exceptionally favorable as a substratum for *C. Anguillulae*, were put in wet soil from local cow pastures, but the desired fungus failed to grow. Butler, J. B. and Buckley (**1927**) found *C. Anguillulae* on the eggs of the sheep liver fluke, *Fasciola hepatica*, in Ireland. They were unable to ascertain the source of infection, but suspected the fungus to be present in tap water from the Dublin water supply, in which the eggs were kept for several months. It would seem, if the *Catenaria* were a common parasite of the sheep fluke eggs, that it should occur in sheep pastures where sheep were infected. To test this hypothesis Dr. J. Wilford Olsen of Angleton, Texas, sent me fluke eggs and soil from a sheep pasture in that state, and Dr. J. N. Shaw of Corvallis, Oregon, sent similar material from Oregon. A tablespoonful of the Texas soil was put into each of six Petri dishes. The soil was banked to one side of the dish, leaving the other side clear, and sterile water was added to the dish. Fluke eggs from Texas were added to the clear side of each dish. Upon examination after two weeks, many of the fluke eggs in each of the six dishes were parasitized by *Catenaria Anguillulae*, and some were parasitized by *Rhizophydium* sp. The soil from Oregon treated similarly, yielded *Hyphochytrium catenoides* Karling, growing within many of the fluke eggs, but so far no *C. Anguillulae* has appeared.



FIGS. 43–48. *Catenaria Anguillulae.*

Catenaria Anguillulae was isolated in absolutely pure culture in the following way. Several infected fluke eggs were sucked up in a small pipet and dropped on a 2 per cent plain agar plate. Using a small needle sharpened to a chisel shape, each infected egg was dragged and pushed about under the binocular dissecting microscope over the agar, until it appeared to be free from bacteria and adhering trash. A block of agar with a single egg was then cut out under the binocular and transferred to agar F_{13}. Several eggs were so isolated, but the fungus grew from only a few. From such a growth several single threads were cut out and each thread transferred to a fresh plate of agar F_{13}. Several of these threads grew, but so slowly that the growth barely kept ahead of the bacteria. However, by cutting threads from the margin, it was possible to establish absolutely pure cultures on agar F_{13}. On this agar growth was slow, a culture attaining a diameter of 2 cms. after 30 days. It seemed desirable therefore, to look for a more favorable culture medium than agar F_{13}.

J. B. Butler and Humphries (**1932**) were the first to grow this fungus in artificial culture. Their best results were obtained in hanging drop preparations, where equal parts of a 0.25 per cent solution of agar in water, a concentrated fluke ova extract in water, and water were used as a growth medium. In this liquid medium with the growth still attached to a fluke egg, a culture about 1 mm. in diameter was obtained (Butler & Humphries, l. c. *pl. 15, fig. 15*).

It occurred to the writer that we might omit the fluke stage entirely and culture the fungus using liver as the source of nourishment. Since no sheep livers were available in the Chapel Hill market, beef liver was used instead. Preliminary tests with beef liver extract agar gave excellent growth. Beef liver agars with and without various sugars were next prepared as follows: a slice of liver (130 gms.) was chopped into small pieces with a razor and put in a towel and ground with a pestle in a mortar. A pinkish purple fluid was squeezed out and water was added to the liver in the towel and this was squeezed through until about 100 cc. of thick liver extract was obtained. Water filtered through blood charcoal was added to the liver extract to make 140 cc. This was boiled for about ten minutes until the proteins coagulated, strained

through a towel, and then divided into seven lots in flasks to which agar and sugars as indicated below were added:

L1—200 cc. liver extract, 1.5 per cent agar—no sugar
L2—200 cc. liver extract, 1.5 per cent agar— 2 grms. maltose
L3—200 cc. liver extract, 1.5 per cent agar— 2 grms. dextrose
L4—200 cc. liver extract, 1.5 per cent agar— 2 grms. sucrose
L5—200 cc. liver extract, 1.5 per cent agar— 2 grms. lactose
L6—200 cc. liver extract, 1.5 per cent agar—10 grms. liver paste
L7—200 cc. liver extract, 1.5 per cent agar—20 grms. liver paste

Petri dishes containing the seven different media were inoculated on the side of the dish. The best growth was obtained on the liver agar without any sugar, but with liver paste added (L7). In this medium the fungus covered the petri dish after about eight weeks, forming a very dense and compact, pale brownish growth, with many sporangia and resting bodies. The stronger the concentration of liver paste, the denser the growth; however, the mycelium spreads more rapidly over the dish in less concentrated liver extract media, but the growth is thin and relatively few reproductive bodies are formed. Of the sugars tested, the only one that promoted abundant growth was sucrose. On this (L4) growth was almost as good as on L6. The growth started slowly, but after about two weeks, the weekly increment was as great as on L7. On liver agar with the other sugars, dextrose, lactose, and maltose, growth was very slow, attaining less than a cm. diameter after eight weeks. However, the growth was very dense and compact. The liver agar is troublesome to prepare and hence I am using a 0.3 per cent meat extract (Difco) in 1.5 per cent agar for stock cultures.

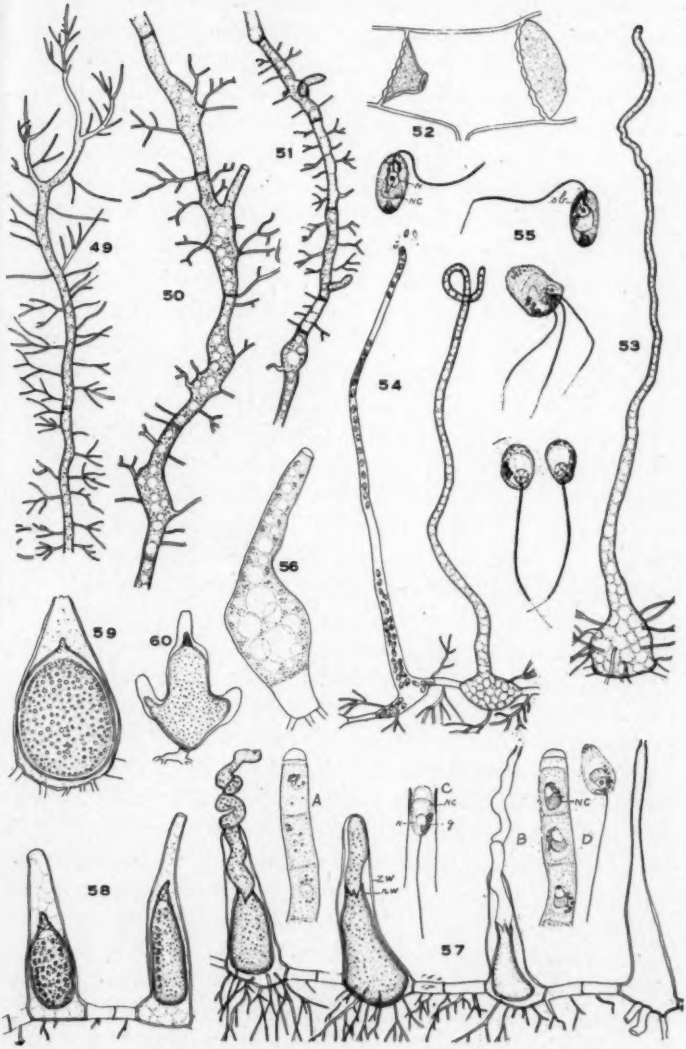
With the fungus in pure culture on agar it was possible to test its ability to grow on various kinds of substrata. However, because of the very extensive food range tests made by Karling (**1934**), it seemed necessary only to test the ability of our strain to grow on nematodes and on plant material. Free living nematodes, collected from branch water were cultured on F_{13} agar. To test the ability of the fungus to infect the nematodes, they were scraped from the surface of the agar and put in water in a Petri dish with zoosporangia and resting bodies of *Catenaria*. Nematodes causing the root knot of the tomato were tested at the same time. The *Catenaria* was able to parasitize both types of nema-

todes (FIGS. 63, 64). The plant material tested was cooked paspalum grass leaves in which the fungus grew, forming both zoosporangia and resting bodies (FIGS. 65–67).

DEVELOPMENT OF THE FUNGUS ON AGAR

The infection stages, the development of the thallus, and the formation of zoosporangia in the liver fluke eggs have been described in detail by Butler, J. B. and Buckley (**1927**). My observations on the development of the fungus in the fluke eggs corroborates theirs. Since the fluke eggs are opaque and the mycelium within them contorted, clear liver agar (as L1) furnishes a far better medium for the observation of the developing fungus. Hence, the observations to follow are made from agar cultures.

The growing end of a hypha tapers to a diameter of about 1 μ (FIG. 49). The rhizoids arise 2–100 μ back of the tip as minute stubs and grow out and branch about through the medium for a distance of a hundred or more microns. The rhizoids are 1–4 μ thick at the base but taper gradually to a fraction of a μ in thickness, retaining always a tubular aspect as indicated by E. J. Butler (**1928**), and by Hillegas (**1940**). The hyphal contents consist of rather coarsely granular, distinctly vacuolate protoplasm. In the growing region, there are no septations, but some distance back from the tip, where the hyphae have attained their mature thickness, septations appear. These are few at first and spaced at irregular intervals. As the hyphae mature, they become more or less regularly septate, the spaces between the cross walls varying from about 60–130 μ long. The swellings which will form the sporangia may start to enlarge either before or after the regular septations are laid down (FIGS. 49–51). Sorokine (**1876**) states, and his figures show, that the septations appear before the swellings, while this is contradicted by Dangeard (**1885**). Butler (**1928**) agrees with Dangeard, stating that the septations are formed after the swellings. Both of these observations are partly correct, since the septations appear both before and after the swellings, but are not so abundant before the swellings as figured by Sorokine. As the swellings are formed, the protoplasm flows into them from both sides until the hypha becomes nearly empty except for the inter-

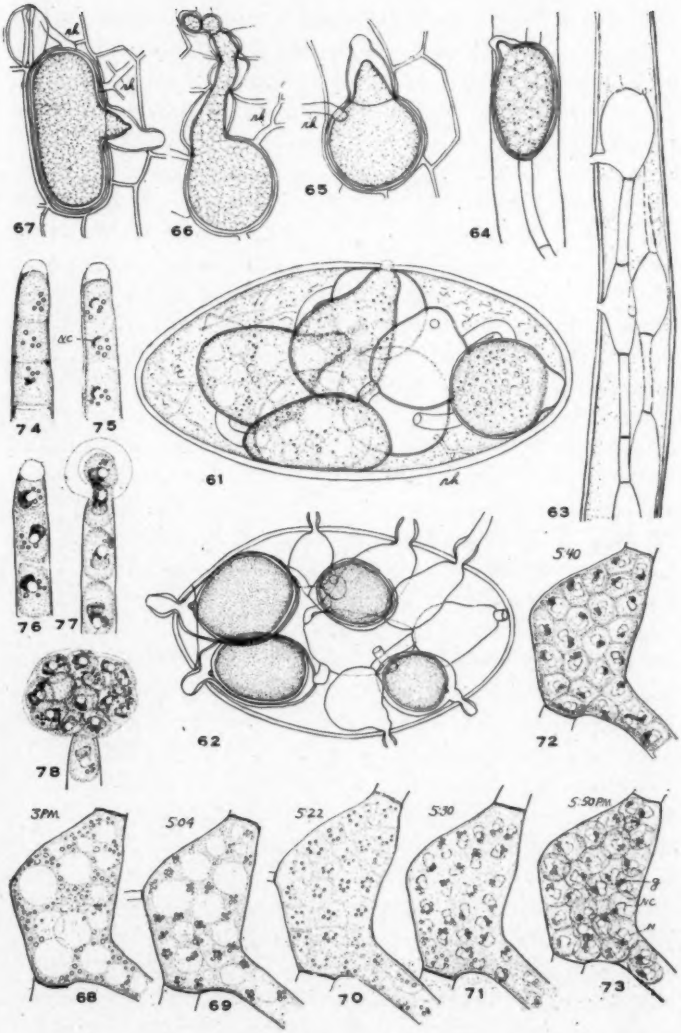


FIGS. 49–60. *Catenaria Anguillulae.*

calary swellings. Cross walls are now formed separating the swollen parts from the unswollen. The swellings become the zoosporangia or resting sporangia, connected by the hyphal isthmuses. These may be once septate or non-septate, depending upon whether the hyphae become septate or not, before the swellings start to form. Sorokine says that the isthmuses are two-celled, *i.e.* once septate, but his figures show them both septate and non-septate.

The development of the sporangium from the time it begins to swell until it attains mature size has not been followed in detail either by previous observers or by me. The observations reported here begin when the sporangium has reached mature size, and end with spore emergence. When mature in size, the cytoplasm contains a large number of spherical vacuoles of variable size and many fat (?) bodies, some of which are 2 or 3 microns thick, but most of which are about 1 μ thick (FIG. 68). While the sporangium is still in the vacuolate stage, the fat globules become arranged in groups of fours or fives and become associated with a rounded body which may be the nucleus (FIG. 69). The clusters are about evenly spaced from each other, and each cluster will be the center of a zoospore and may at this stage show a slight rocking motion.

The vacuoles disappear rather suddenly and though they are for the most part large and conspicuous, I am still uncertain about their fate. By concentrating observations on one large vacuole, something has been learned. The outline became irregular and vague and in a few seconds had disappeared. All vacuoles disappeared almost simultaneously, and immediately cleavage furrows became evident, the furrows apparently forming from the material contained within the vacuoles (FIG. 70). If this were so, it would furnish evidence that the vacuolar material was cytoplasmic in nature and hence living, and that the cleavage process was not an osmotic or turgor process alone, but one involving the rearrangement and redistribution of the vacuolar material in between the spore origins. In fifteen to twenty minutes after the vacuoles disappeared, the contents of the sporangium were divided into polygonal areas, each a spore origin with a cluster of 4 to 8 globules (FIG. 70). After about thirty minutes, the cleavage furrows between the spore origins disappeared and soon a greenish hyaline material began to take on a half-moon shape on one side of the nu-



FIGS. 61–78. *Catenaria Anguillulae.*

cleus (FIG. 71). This is the nuclear cap. The spores now reformed and became polygonal again (FIG. 72). While in this shape the spores showed considerable motion. A single spore might spin around, but retained its polygonal shape and position in the sporangium. Shortly before emerging, *i.e.* two or three minutes, the spores became round and began a rocking and trembling motion (FIG. 73).

Meanwhile changes had taken place in the wall substance of the tip of the emergence papilla. This thickened until it took on the appearance of a convex lid (FIG. 74). A few minutes before spore discharge, this region gelatinized, the gelatinous material pushing backward in the tube, forcing the spores back (FIGS. 75, 76). In several instances the outer part of the gelatinizing tip broke away as a fairly distinct lid. Suddenly the gelatine expanded outwards into a spherical, hyaline ball, the spores now moving outwards as though pushed by some expanding, intersporal substance (FIG. 77). The first spore pushed out into the center of this hyaline sphere and others followed rapidly to form a spherical ball of spores (FIGS. 77, 78). At first the spores were quiet, but in a few seconds they became active; the envelope burst and the spores which were in the ball swam away. The remaining spores emerged singly and swam away as they reached the exterior. While it seems the rule for the first spores to be discharged into a spherical mass surrounded by hyaline material, this is by no means always the case, for often the tip gives way and the first as well as the later spores swim away upon reaching the exterior. Sometimes the first spores to emerge may collect into an irregular mass, swimming away one by one as they become disentangled. Thus in perfectly normal and uninjured material one may find at different times all the variations in spore discharge which have been described by Sorokine, Dangeard, and others, except that in no instance have I observed spores discharged without cilia. The above observations confirm those of Dangeard (**1885**) and Karling (**1938**). One interesting variation noted by Karling (**1938**) related to the discharge of multiciliated masses of protoplasm from the zoosporangia. As observed by him, these may swim about in uncoordinated fashion or they may creep about like an amoeba. Some of these masses absorbed water and swelled to more than a hundred microns in diameter. On

such monsters the ends of some of the cilia became swollen to form a vesicle. This is excellent evidence that the cilium is a tubular structure with a core of perhaps vacuolar material. Structures which look like vesicles have been referred to by Berdan (**1941**) as loops. The zoospores as in *Catenaria Allomycis* have the structure (FIGS. 47, 55) and method of swimming characteristic of the Blastocladiales.

VALIDITY OF SPECIES OF CATENARIA

The fungi which have been described under the name of *Catenaria Anguillulae* certainly represent more than one species, as has been suggested by several authors and probably do not all even belong to the same genus or family. Indeed it is impossible to be certain as to Sorokine's type, since it was inadequately and perhaps partly incorrectly described. On the other hand, it seems highly likely that the fungi identified by Dangeard (**1885**), Constantineau (**1901**), J. B. Butler and Buckley (**1927**), E. J. Butler (**1928**), Buckley and Clapham (**1929**), J. B. Butler and Humphries (**1932**), and Karling (**1934**) as *Catenaria Anguillulae* are all one and the same species and identical with the species described here. It has been pointed out by Dangeard and others that the chief apparent differences between Sorokine's fungus and theirs is in the abundance of rhizoids, and the smaller dimensions of the sporangia and zoospores in Sorokine's fungus. I was able to infect nematodes with inoculum from a pure culture of my strain of *C. Anguillulae* (FIGS. 63, 64). On the nematodes my fungus agreed remarkably well with Sorokine's. It is easy to understand how Sorokine might have overlooked the rhizoids with a magnification of 450 diameters, for I find they are difficult to see in a nematode even with a 70 × water immersion objective. Sorokine gives the sporangia as 8–10 × 10–17 μ and the spores as 1.5–2 μ thick. It is possible, indeed likely, that these figures are wrong, for if one calculates the size of the sporangia and spores from Sorokine's drawings, the sporangia are 10–26 × 20–53 μ, the largest one shown (Sorokine, l. c. FIG. 24, sporangium to right) is 26 × 53 μ; the zoospores are about 4 × 6 μ. Sorokine incorrectly describes the cilia as forming after the spores emerged, and states that the spores

have a single, conspicuous globule which he calls a nucleus. These are doubtless errors of observation due to the low magnification used. He correctly notes that the movement of the spores of *Catenaria* does not resemble that of the spores of the chytrids. Sorokine says that the spores emerge one by one or by twos, threes, or indeed they may emerge in a mass separating immediately after their exit to swim away. After the sporangium becomes partly empty, the spores remaining in the sporangium begin moving about.

Dangeard (**1885**) first suggested that the organism described by Villot (**1874**) as a fresh water alga parasitic on the hair worm, Gordius, was in reality the fungus *Catenaria Anguillulae*. Villot was of the opinion that more than one species of fresh water alga [*Catenaria*] was involved. On pages 181 and 182 Villot describes the methods used in studying the structure of the hair-worms. Free hand sections were made of fresh worms and the sections were cleared in a mixture of acetic acid, 1; alcohol, 1; glycerine, 1; distilled water, 2. Staining with carmine was found useful. It was apparently in material so treated that the fungus was observed. Such treatment probably accounts for the collapse of the contents of the sporangia in figure 13. Villot states that the zoospores are spherical, 2 μ thick and without cilia. He says that the spores occur in great numbers in suspension in the water of the streams. It is very likely that Villot had the spores of some other organism. In figure 14, he shows what he calls a zoosporangium. This is undoubtedly a typical resting body, so well drawn that it shows the characteristic papilla shown by Buckley and Clapham (**1929**) and myself. Indeed I regard this resting body as the best evidence that Villot's fungus belonged to the genus *Catenaria*. Villot gives the diameter of the hyphae as 2 μ thick, which is about half the diameter of the smallest hyphae I have seen. The thickest isthmus shown in figure 13 is slightly more than 4 μ thick; those in figures 14 and 16, 2.5 and 2 μ thick respectively. The largest sporangia shown, figure 16, are about 15 $\times$ 25 μ which agree well with the sizes of Sorokine's, according to my calculations.

Karling (**1934, 1938**) showed that *C. Anguillulae* would grow on a very wide range of plant and animal material. The strain reported on in this paper grows on nematodes, fluke eggs, and

various other substrata. I hope to try it on Gordius when I can find such worms. Until this is done, we must remain in doubt as to the specific identity of Villot's fungus.

The fungus described and illustrated by Sparrow (**1932**) as *C. Anguillulae* is certainly not the same as *C. Anguillulae* in the sense of Dangeard and as interpreted here. The spores of Sparrow's fungus were 2 μ in diameter, spherical, and with one or two oil globules. If the spore is correctly described, this fungus would hardly seem to belong to the genus *Catenaria*. It must be regarded as a fungus whose relationship is doubtful.

Karling (**1942**, p. 622) reported the resting bodies of *C. Anguillulae* in material collected in Cypress Gardens, South Carolina. He states that they are spherical, oval, oblong, and full of yellowish-amber, refractive material, and have a fairly thick, hyaline wall. In my material, the wall of the resting body is pale brown in color and the contents hyaline. Even when the main part of the spore is spherical, it always has a short papilla or "pip" on its surface beneath the old empty tube. Since Karling does not mention this very characteristic pip, and the color of the resting bodies mentioned by him does not correspond with that of *C. Anguillulae*, it is doubtful if the resting bodies from South Carolina belong to *C. Anguillulae* as interpreted here.

Serbinow's *Catenaria pygmaea* has been regarded as a doubtful species of *Catenaria* by von Minden (**1911**), E. J. Butler (**1928**), and others. In this species the thallus is monocentric, the zoospores 1.5 μ thick, with a single fat body, and the resting bodies are spherical, having a smooth colorless membrane with a central fat body, characters which should certainly exclude Serbinow's species from the genus.

Karling's (**1928**) *Catenaria sphaerocarpa* is distinguished primarily by the spherical zoospores with a single conspicuous refractive globule, the zoospores swimming as in the monocentric chytrids; by the usually spherical resting spores with a thick brown wall, and the predominantly spherical zoosporangia. The structure of the zoospores is of such basic and fundamental importance in determining relationships that on this character alone *C. sphaerocarpa* should not be included in *Catenaria* as interpreted here.

We are thus left with only one species in the genus *Catenaria; C. Anguillulae* in the sense of Dangeard, the two Butlers, Buckley and Clapham and others (see p. above). As pointed out above, we can never know for certain if Sorokine's type is the same as the fungus described by Dangeard, Butler, and others, unless someone can rediscover Sorokine's fungus from the type locality. However, I think the evidence is in favor of the view that Sorokine's and Villot's fungi belong in the same genus with Dangeard's and mine. Whether or not all three are the same species can only be determined with certainty by further study of the parasites of Gordius and eel-worms. For the present, I think it prudent to follow a suggestion made by Karling (**1938**) and to retain the name *C. Anguillulae* for the fungi studied by Villot, Sorokine, Dangeard, Constantineau, J. B. Butler and Buckley, E. J. Butler, Buckley and Clapham, Butler and Humphries, Karling, and myself.

The following description drawn up from the material from Texas, allows sufficient latitude for the inclusion of all the above forms, if one makes sufficient allowance for the measurements of Villot and Sorokine and some of the perhaps faulty observations of the latter.

CATENARIA ANGUILLULAE Sorokine.

Parasitic or saprophytic in nematodes, liver fluke eggs, and saprophytic on cooked grass leaves, etc., and various kinds of nutrient agar. Thallus composed at first of a branched or unbranched nonseptate or sparingly septate hypha, with rhizoids. Hypha 4–15 μ thick, swelling at more or less regular intervals to form zoosporangia or resting bodies connected by narrow one or two celled isthmuses and thus catenulate. Zoosporangia pyriform or subpyriform in fluke eggs, 25–36 × 38–71 μ or considerably smaller when crowded, emergence papillae 5–8.6 × 8–several hundred microns long, connecting isthmuses 5–5.4 × 4–14 μ; in nematodes zoosporangia oval or elliptic, 9–20 × 12–34 μ, the emergence papilla only projecting through the nematode skin; on liver agar zoosporangia subglobose with very long emergence tubes, up to a mm. long; zoospores completely formed within the sporangium, usually showing rocking motion before discharge; first spores usually emerging to form a spherical mass enclosed by a gelatinous substance at the tip; this gelatinous envelope soon dissolves and the first spores swim away, the rest of the spores swim away as soon as they reach the exit, or first spores may swim away im-

mediately; zoospores 3.8–5.4 × 6.7–8 μ, tapering toward the anterior end, with 3–4 anterior fat (?) globules, a distinct nuclear cap and nucleus to one side of which is a side body and several fat? globules; with one posterior whiplash cilium; rounding up and encysting before germinating, 4.6–5.4 μ; germinating in water or nutrient agar by sending out a delicate rhizoid and then forming a tubular growth from the opposite pole, which may form a dwarf sporangium with a small, sterile basal part, or may grow into a new mycelium if sufficient food is available [on fluke eggs, zoospore leaves empty cyst on outside of egg membrane (Butler & Buckley, **1927**)]. Resting bodies formed on nematodes, fluke eggs, leaf tissue, and nutrient agars; formed within zoosporangial membrane and conforming somewhat to its shape, resting spore protoplasm retreating from the old sporangial wall and forming a new thick pale brownish wall of its own; on nematodes oval or oblong ovate, 16–18 × 20–33 μ, in fluke eggs spherical, subspherical, ovoid or irregular in shape, 21–42 μ when spherical, 20–33 × 40–55 μ when subspherical; on boiled leaves spherical, pyriform, lobed, or cylindrical with rounded ends frequently conforming to the leaf cells except for a cone-shaped part through which the emergence tube sprouts, 30–50 × 38–100 μ; on agar spherical or subspherical, except for a small or large cone-shaped elongation up to over 100 μ long, on agar No. 5 up to 138–176 μ thick when spherical or subspherical; wall smooth except for a short papilla apparently always present and always directed towards emergence pore of zoosporangium, wall about 2–3 μ thick. Resting sporangium germinating by the irregular cracking of the outer thicker wall and the emergence of a long or short tube through which the zoospores emerge as in the zoosporangium. Zoospores in resting sporangia as in zoosporangia.

Isolated by the writer from sheep liver-fluke eggs from Texas, which were put in wet soil collected from a sheep pasture near Angleton, Texas, May, 1944. Soil and fluke eggs collected by Dr. J. Wilford Olsen, May, 1944.

Sparrow (**1943**) recognized the Catenarioideae as a subfamily in the Chytridiales. In view of the studies presented here it seems advisable to transfer the genus to the Blastocladiales as a family.

Catenariaceae fam. nov.

(Subfam. Catenarioideae Sparrow)

Fungi in vermibus et in aliis fungis parasitici, vel in materia plantarum et animalium saprophytici. Thallus immaturus tubulatus, maturus catenulatus

cum rhizoideis paucis vel numerosis. Zoosporae omnibus rebus similes illis Blastocladialium. Cellulae perdurantes ut in Blastocladialibus efformatae, pariete levi vel minute punctato fulvoso; protoplasmate hyalino. Zoosporae ex cellulis perdurantibus pusillos thallos gignentes aut in sporas immotas (gametangia) se convertentes et quattuor cellulas sexuales formantes quae per paria copulant et biflagellatas zygosporas formant.

Parasitic on worms, other fungi or saprophytic on a great variety of plant and animal substrata. Thallus worm-like, unbranched or sparingly branched, with a few to many rhizoids, swelling at more or less regular intervals to form the reproductive organs which are zoosporangia or resting bodies connected by one or two-celled narrow isthmuses. Zoospore formation, discharge, and structure as in the Blastocladiales. Resting bodies formed within the zoosporangial or hyphal membrane usually contracting from the membrane to leave a more or less conspicuous space. Wall of resting body smooth or very minutely pitted or with a single pip, pale brown, contents hyaline. In germinating the resting body is transformed into a sporangium, the outer wall cracks irregularly and through this crack a tube of variable length emerges. Zoospores from resting bodies uniciliate and acting as zoospores which give rise to dwarf thalli or encysting and acting as gametangia to form four gametes which fuse in pairs to form a biciliate zygote.

What is the justification for the transference of *Catenaria* from the Chytridiales to the Blastocladiales? Sorokine (**1876**) suggested that *Catenaria* resembled *Achlygeton* in its development. Dangeard (**1885**) placed it tentatively in the Ancylistales, but Fischer (**1892**), Schröter (**1897**), von Minden (**1911**), Fitzpatrick (**1930**), Karling (**1932**), Sparrow (**1943**), Whiffen (**1944**) have put it in the polycentric Chytridiales. Sparrow (**1943**), recognizing the peculiar thallus of *Catenaria* when compared with the chytrids, put the genus in a subfamily, the *Catenarioideae*. Karling (**1931**) in his review of *Catenaria* points out that the method of formation of the resting bodies is quite unlike that of other species of the *Cladochytriaceae* and follows Dangeard in suggesting a possible relationship to the Ancylistales.

The reasons for putting *Catenaria* in the Blastocladiales may be summarized as follows:

1. The zoospore structure is similar to the structure of the spores in the Blastocladiales, with numerous fat bodies, a side body, and a nuclear cap.

2. Zoospore discharge is typical of the Blastocladiales, *i.e.* the first zoospores to emerge collect in a ball at the sporangial tip as in *Blastocladiella, Allomyces,* etc.

3. The zoospore swims as in the Blastocladiales.

4. Zoospore germination is usually as in the Blastocladiales, *i.e.* bipolar instead of monopolar. First noted by Dangeard, 1885.

5. The resting body is formed in a hyphal or zoosporangial case as in the Blastocladiales, and germinates as in that order and not as in any chytrid.

6. Septations are frequently irregular, *i.e.* bumpy, ridged or apparently pitted.

7. Life cycles parallel certain Blastocladiales. *Catenaria Anguillulae* has a life cycle as in *Allomyces anomalus* or *Blastocladiella simplex, laevisperma,* or *asperosperma; C. Allomycis* as in *Allomyces cystogenus.*

From the above it is clear that *Catenaria* is closer to the genera *Blastocladiella* and *Allomyces* than to any other fungi. Indeed one might consider *Catenaria* as a sort of polycentric *Blastocladiella* though I do not think either of these fungi arose from the other. The catenulate thallus is the chief basis justifying the establishment of a separate family, but in *Allomyces moniliformis* one frequently finds the zoosporangia and resting bodies connected by isthmuses as in *Catenaria* (FIG. 12).

SUMMARY

A fungus with a thallus similar to *Catenaria Anguillulae* and resting bodies that germinate as in *Blastocladiella cystogena* has been isolated from soil with its host, *Allomyces anomalus.* Growth experiments indicate that it is an obligate parasite on species of *Allomyces* and *Blastocladiella simplex.* It failed to grow on *B. cystogena, B. laevisperma,* or *B. asperosperma,* or on *Blastocladia parva, Achlya* sp. or *Saprolegnia* sp. Infection stages on *Allomyces anomalus,* the development of the thallus, zoosporangia, resting bodies, and the fusion of motile gametes are described. The fungus is named *Catenaria Allomycis. Catenaria Anguillulae* has been isolated from Texas soil and grown in pure culture on a variety of agars for the first time. It grows best on beef liver agar

with or without sucrose, on which it forms a much branched thallus with numerous rhizoids. It can be transferred to liver fluke eggs, adult nematodes, and boiled grass leaves. Zoosporangia are formed in nearly all culture media tried. Resting bodies are formed sparingly in liver fluke eggs, nematodes, grass leaves, but are formed abundantly in strong concentrations of liver agar. The resting bodies are formed and germinate much as in *Blastocladiella*. On the bases of zoospore structure, method of discharge, and swimming and resting body structure and germination, the genus *Catenaria* is transferred from the Chytridiales to the order Blastocladiales, and Sparrow's subfamily *Catenarioideae* is raised to family rank. The species *Catenaria Allomycis* shows a parallel in its life history to *Allomyces cystogenus* while the life cycle of *C. Anguillulae* is similar to that of *A. anomalus*.

UNIV. OF NORTH CAROLINA,
CHAPEL HILL, N. C.

LITERATURE CITED

Barrett, J. T. 1912. Development and sexuality of some species of *Olpidiopsis* (Cornu) Fischer. Ann. Bot. **26**: 209–238.

Berdan, Helen Berenice. 1941. A developmental study of three saprophytic chytrids. II. *Catenochytridium carolinianum* Berdan. Am. Jour. Bot. **28**: 901–911.

Buckley, J. J. C. & Phyllis A. Clapham. 1929. The invasion of Helminth eggs by chytridiacean fungi. Jour. Helminthology **7**: 1–14.

Butler, E. J. 1928. Morphology of the chytridiacean fungus, *Catenaria Anguillulae*, in liver-fluke eggs. Ann. Bot. **42**: 813–821.

Butler, J. B. & J. J. C. Buckley. 1927. *Catenaria Anguillulae* as a parasite of the ova of *Fasciola hepatica*. Sci. Proc. Royal Dublin Soc. **18**: 497–512.

—— **& Annie Humphries.** 1932. On the cultivation in artificial media of *Catenaria Anguillulae*, a chytridiacean parasite of the ova of the liver fluke, *Fasciola hepatica*. Sci. Proc. Royal Dublin Soc. **20**: 301–324.

Constantineau, J. C. 1901. Contributions à la flore mycologique de la Roumanie. Rev. Génér. Bot. **13**: 389.

Couch, J. N. & Alma J. Whiffen. 1942. Observations on the genus *Blastocladiella*. Am. Jour. Bot. **29**: 582–591.

Dangeard, M. 1885. Note sur le *Catenaria Anguillulae* Sorok. et *Chytridium zootocum* A. Br. Bull. Soc. Linn. Normandie IIIe. **9**: 126–135.

Fischer, Alfred. 1892. Phycomycetes. Rabenhorst's Kryptogamenflora, 1, IV, Abt. Kummer, Leipzig.

Fitzpatrick, H. M. 1930. The Lower Fungi. Phycomycetes, pp. 1–331. New York.

Hillegas, Arthur B. 1940. The cytology of *Endochytrium operculatum* (de Wildeman) Karling in relation to its development and organization. Bull Torrey Club **67**: 1–32.

Karling, J. S. 1931. Studies in the Chytridiales VI. The occurrence and life history of a new species of *Cladochytrium* in cells of *Eriocaulon septangulare*. Am. Jour. Bot. **18**: 526–557.

——. 1934. A saprophytic species of *Catenaria* isolated from roots of *Panicum variegatum*. Mycologia **26**: 528–543.

——. 1938. A further study of *Catenaria*. Am. Jour. Bot. **25**: 328–335.

——. 1942. A new chytrid with giant zoospores: *Septochytrium macrosporum* sp. nov. Am. Jour. Bot. **29**: 616–622.

Schröter, J. 1897. Chytridiineae. Engler and Prantl, Die Natürlichen Pflanzenfamilien, **1**, 1. pp. 64–87. Leipzig.

Sorokine, N. 1876. Note sur les végétaux parasites des *Anguillulae*. Ann. Sci. Nat. VI°. **4**: 62–71.

Sparrow, F. K. (jr.). 1932. Observations on the aquatic fungi of Cold Spring Harbor. Mycologia **24**: 268–301.

——. 1943. Aquatic Phycomycetes. pp. 318–320. Ann Arbor.

Villot, A. 1874. Monographie des Dragonneaux. Arch. Zoologie expérimentale et générale **3**: 184–185.

Von Minden. 1911. Chytridiineae. Kryptogamenflora der Mark Brandenburg. I. **5**: 390–419. Leipzig.

Whiffen, Alma, J. 1944. A discussion of taxonomic criteria in the Chytridiales. Farlowia **1**: 583–597.

EXPLANATION OF FIGURES

FIGS. 1–11. *Catenaria Allomycis.* The host is *Allomyces anomalus.* 1, hypha of host with short half mature parasite, × 100; 2, heavily infected branched hypha showing spherical resting bodies and empty zoosporangia of parasite, × 100; 3, dichotomously branched hypha of parasite in host, × 100; 4, zoosporangia of host and nearly mature hypha of parasite, × 100; 5, two empty zoosporangia and resting bodies of parasite, note septations, × 100; 6, germinating resting bodies of parasite, note long tube on right and two loose clusters of cysts near center, × 150; 7, germinated resting body showing irregularly cracked wall, emergence tube, and several encysted resting-body zoospores, other zoospores are out of field, × 700; 8, empty cysts and one with two or three gametes, × 1400; 9, empty cysts and gametes emerging, × 1050; 10, zygote stained with two cilia separated, × 1400; 11, same with two cilia together, × 1400.

FIGS. 12–31. *Catenaria Allomycis.* Fig. 12, on *Allomyces moniliformis,* all others on *Allomyces anomalus.* 12, zoosporangia of *Allomyces moniliformis* showing connecting isthmuses, × 127; 13–20, stages in invasion of host by parasite, × 1244; 21–24, stages in development of parasite from small

oval body to the catenulate thallus with rhizoids and septations, × 564; 25, dwarf thallus forming single sporangium in exhausted host thread, × 675; 26–30, development and discharge of zoospores, see text for explanation, × 675; 31, zoospores killed and stained, drawn while wet; n.c., nuclear cap; n, nucleus; s.b., side body; g, lipoid granules or fat bodies, × 1244.

FIGS. 32–42. *Catenaria Allomycis.* Host *Allomyces anomalus.* 32, 33, habit sketches showing empty zoosporangia and resting sporangia in thread of *Allomyces,* × 235; 34, resting sporangia and rhizoids in thread of *Allomyces,* × 800, small insert lower right shows perforated septum of parasite, × 1120; 35, wall of resting sporangium cracked and germ tube growing out through crack and host wall, × 508; 36, distal part of tube of same resting sporangium, but later, showing r.s. zoospores forming, note distinct circles of fat bodies, × 508; 37, r.s. zoospores emerging, some swimming sluggishly, others encysted, × 508; 38, cysts with papillae formed, × 608; 39, cysts some empty at tip of emergence tube of resting sporangium, note one cyst (?) germinating by tube, × 294; 40, habit sketch of germinating resting bodies of *C. Allomycis* with extra long tubes, note distinct and very characteristic circles of fat globules, a "circle" for each spore, on left a cluster of cysts, on right gametes emerging from cysts, g, gamete, z, zygote, × 152; 41, emergence of gametes from cysts and fusion of gametes in living material, × 1120; 42, two zygotes, killed with fumes of osmic acid and stained with gentian violet, × 1120.

FIGS. 43–48. *Catenaria Anguillulae.* 43, vertical section through agar No. 5 showing resting bodies and zoosporangia, × 200; 44, in 0.3 per cent meat broth extract and then in charcoal water, zoosporangia and rhizoids, × 100; 45, on 0.3 per cent meat extract agar + 1 per cent dextrose, × 150; 46, resting body from agar No. 5, × 700; 47, on 0.3 per cent meat extract agar, note rhizoids have blunt tips, × 400; 48, on F_{18} agar, sector on right with resting bodies, on left without, × 100.

FIGS. 49–60. *Catenaria Anguillulae.* 49–51, hyphae on liver agar; 49, distal part of hypha showing 2 septations before swellings start; 50, septations separating swellings, end walls delimiting sporangia from isthmuses will form later; 51, sporangia forming with a minimum of swelling, × 224; 52, cross walls in hypha showing pits, × 1065; 53, 54, zoosporangia with long tubes, from culture in meat extract broth transferred to charcoal water, spores emerging in sporangium on left, × 137; 55, stained material showing four normal and 1 giant spore with 3 cilia, two lower spores dried over night, nuclear cap appeared as empty space, s.b., side body; n, nucleus; n.c., nuclear cap, × 1065; 56, dwarf zoosporangium in water cultures with boiled grass but not attached to it, × 577; 57, three resting sporangia in stage of germination and one empty zoosporangium on right, × 224; z.w., zoosporangial wall; r.w., resting sporangial wall, on L7 agar. Insert A, zoospores in tip of tube about 15–20 minutes before discharge, nucleus and fat bodies distinct, tip beginning to gelatinize; B, 15 minutes later nuclear cap (n.c.) formed, tip gelatinizing; C, spore emerging cilium trailing; D, diagram of spore in swimming condition, A–D, × 1065; 58, two resting sporangia on L7 agar, note cytoplasm between resting body and zoosporangial wall, × 224; 59, resting sporangium, on L3 agar, × 224; 60, lobed resting sporangium, on L3 agar, × 137.

FIGS. 61–78. *Catenaria Anguillulae* Sorokine. 61, 62, on liver fluke eggs from Texas in soil from Texas; 61, shows three immature zoosporangia and one immature resting sporangium, several empty zoosporangia, indistinct rhizoids and disintegrated contents of fluke egg, note emergence pore of zoosporangia; 62, several empty zoosporangia showing emergence tubes and four resting sporangia, × 445; 63, 64, on nematode sp.; 63, empty zoosporangia, × 445; 64, resting sporangium, × 445; 65–67, resting sporangia in boiled leaf, paspalum grass, × 280; 68–73, zoosporangial development, see text for explanation, n, nucleus; n.c., nuclear cap; g, fat globules, × 760; 74–78, distal part of zoosporangial tube, see text for explanation, × 1065.

NOTES ON THE CULTURE OF COPRINUS ASTEROPHORUS[1]

HORACE L. BARNETT

(WITH 7 FIGURES)

In the spring of 1942, four fruit bodies of a species of *Coprinus* were collected in sandy soil by a roadside near Mesilla Park, New Mexico. The specimens were dried and stored in the laboratory. Interest in this brief study of this fungus was stimulated by Dr. W. H. Long, who saw the specimens and identified them as belonging to an unnamed species. It has recently been described and named *Coprinus asterophorus* Long and Miller.[2]

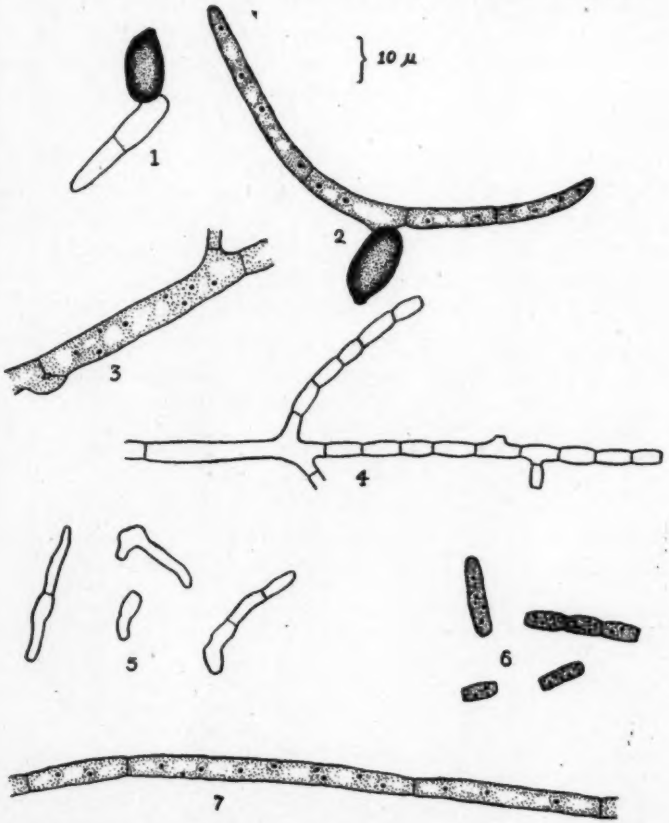
In the fall of 1942 the spores from a dried fruit body were sown on potato-dextrose agar plates. Germination was slow, beginning after 24 hours, with only a small percentage of the spores germinating. A single germ tube appears at the end opposite the apiculus (FIG. 1) and soon branches, commonly forming a two-pronged structure (FIG. 2). Soon, numerous branches are produced. No clamp connections are present on the young mycelium.

The growth of the mycelium at room temperature is fairly rapid. Macroscopically, the mycelium is pure white, much of it being aerial, and has a fluffy tufted appearance. After several days, microscopic examination revealed the presence of clamp connections on both the submerged and aerial portions of the mycelium (FIG. 3), being more frequent on the latter, but not very abundant on either. Oidia were present on the aerial tufts. The oidia are formed by the segmentation of some of the upright hyphae (FIG. 4). They are rod-shaped and range from 5 to 15 μ in length. On agar the oidia germinate by one or two germ tubes (FIG. 5) and produce normal mycelium with clamp connections.

[1] The investigations on which this article is based were completed at New Mexico College of Agriculture and Mechanic Arts, State College, New Mexico.

[2] Long, W. H. & Vera Mentzer Miller. *Coprinus asterophorus*, a new desert *Coprinus*. Mycologia 37: 120. 1945.

Single germinated basidiospores were picked out with a needle and cultured. The resultant mycelia were, from all appearances, like those which arose from many spores. After 10 days, the cul-



FIGS. 1 and 2, basidiospores germinating on agar; 3, portion of older mycelium showing clamp connection and nuclei; 4, portion of aerial hypha showing the formation of oidia; 5, oidia germinating on agar; 6, oidia showing nuclei; 7, portion of mycelium showing cells with varied number of nuclei. Figs. 1, 4 and 5 are from unstained material.

tures were examined microscopically. All of the 17 single spore cultures obtained produced clamp connections and oidia. Thus, the species is homothallic.

All attempts to stimulate the production of fruit bodies in pure culture failed.

Germinated basidiospores and mycelium were stained with Heidenhain's Iron-alum Haematoxylin. As far as could be determined, the first cell of the germ tube is binucleate, although only a few germ tubes in this stage were observed. Since the spore wall is very dark and only mature spores were available, it could not be determined whether the basidiospore contains one or two nuclei.

The cells of the mycelium contain two or more nuclei (FIGS. 3, 7). This is true for mycelium resulting from a single spore as well as that resulting from many spores. The number of nuclei in one cell is more often even, but not constantly so. In general, the longer cells contained the larger number of nuclei.

Most of the oidia were found to contain either two or four nuclei (FIG. 6), although a few uninucleate oidia were seen. The binucleate condition seemed to be typical. Those containing four nuclei often appeared to break up into two binucleate oidia.

SUMMARY

Coprinus asterophorus is a homothallic species, producing clamp connections and typically binucleate oidia which germinate to produce typical mycelium with multinucleate or binucleate cells.

DEPARTMENT OF BOTANY,
STATE UNIVERSITY OF IOWA,
IOWA CITY, IOWA

THE GENUS LAMPRODERMA AND ITS RELATIONSHIPS. II

MARY LOUISE DENNISON

(WITH 1 FIGURE)

STEMONITACEAE

Spores in mass black, deep violaceous or ferruginous; capillitium always present; peridium and capillitium limeless; lime, if present, restricted to hypothallus, stipe and columella; fructifications aethalioid or of separate and distinct, globose to cylindric, sessile to stipitate sporangia; columella varying from almost none to very prominent; capillitium typically abundant, composed of rather slender, freely branching and anastomosing threads arising from the columella or base of the sporangium, the tips free, attached to the peridium or united to form a surface net; peridium evanescent to persistent.

In the Stemonitaceae as proposed the following genera will be included: *Schenella* (?), *Amaurochaete, Brefeldia, Diacheopsis, Diachea, Elaeomyxa, Macbrideola, Enerthenema, Clastoderma, Barbeyella, Lamproderma, Stemonitis,* and *Comatricha.*

Type Genus: *Stemonitis* Gleditsch emend. Rost.

KEY TO GENERA

a. Fructification aethalioid ..... b
a. Fructification sporangiate ..... d
  b. Capillitial threads unbranched, united into columnar cords and attached both at base and peridium; columella lacking ..... 1. *Schenella*
  b. Capillitium branched, dendroid c
c. Columellae obscure or lacking, capillitium not vesicular .. 2. *Amaurochaete*
c. Columellae distinct above, blending beneath; capillitium vesicular ..... 3. *Brefeldia*
  d. Columella obscure or lacking ..... 4. *Diacheopsis*
  d. Columella present (lacking in *L. insessum*) ..... e
e. Hypothallus, stipe and columella calcareous ..... 5. *Diachea*
e. Hypothallus, stipe and columella free from lime ..... f
  f. Stipe and columella and sometimes capillitium and peridium waxy ..... 6. *Elaeomyxa*

f. Stipe and columella neither waxy nor calcareous .....g
g. Capillitium lacking or replaced by a few branches at tip ...7. *Macbrideola*
g. Capillitium well developed .....h
h. Columella percurrent; capillitium arising from a disk at its apex .....8. *Enerthenema*
h. Columella rarely percurrent; capillitium arising from the entire length of the columella or if from the apex, not from a disk i
i. Capillitium brush-like, rigid, ultimate branches bearing at their tips circular disks derived from the peridium .....9. *Clastoderma*
i. Capillitium without conspicuous circular disks at tips of rigid branches .....j
j. Peridium persistent, iridescent .....k
j. Peridium fugacious l
k. Peridium dehiscent into irregular persistent petaloid lobes; capillitium of simple dark threads .....10. *Barbeyella*
k. Peridium as a whole persistent; capillitium freely branching and anastomosing .....11. *Lamproderma*
l. Tips of capillitial branches united to form a more or less complete surface net .....12. *Stemonitis*
l. Tips of capillitial branches not united to form a surface net .....13. *Comatricha*

Stemonitis Gleditsch 1753 is the type of the family Stemonitaceae. In its modern application, it is characterized by distinct or fasciculate, stalked, cylindrical sporangia with a prominent columella usually extending the entire height of the sporangium, or nearly so, and giving rise on all sides to the capillitium which forms a surface net by anastomosis of the many capillitial threads. The peridium of this genus is very evanescent.

The genus *Comatricha* was segregated from *Stemonitis* by Preuss in 1851. The primary character on which the segregation was based is the lack of a surface net in *Comatricha*. The anastomosis of the capillitial filaments in *Comatricha* is general from the columella to the periphery of the sporangium, with the ultimate tips free, not supporting a surface net. In *Stemonitis* the anastomosis of the capillitial filaments ends in the formation of a surface net just beneath the fugacious peridium. Occasionally an imperfectly developed surface net may be observed in species of *Comatricha;* in *Stemonitis,* where a capillitial net is characteristic, it is usually imperfectly developed in *Stemonitis nigrescens* and *S. hyperopta.* The sporangia of *Comatricha* vary in shape from globose to ovoid to cylindrical, the columella extends into the sporangium at least

half-way, bearing branches on every side, and the peridium is more persistent than in *Stemonitis,* sometimes remaining at the base, as in *C. Rispaudii* and *C. cornea* or, in some collections of *C. typhoides,* persistent throughout.

Since there are species of both these genera which show varying degrees of intergradation, the separation may be regarded as useful rather than significant.

The genus *Lamproderma,* which has been critically discussed in the preceding paper, exhibits characters common to both *Stemonitis* and *Comatricha,* but is a little more specialized. In discussing the relationships of *Lamproderma* with the other genera of the family it might not be amiss to regard *Comatricha* as the center for comparison with *Stemonitis* on the one hand, characterized by cylindrical sporangia, fugacious peridium and a long conspicuous columella giving rise on all sides to the capillitium, at the tips of which a conspicuous surface net is formed, and *Lamproderma* on the other, with its globose to ovate sporangia, its rather persistent, iridescent peridium and its branching and anastomosing capillitium arising mainly from the apex of a shorter columella; these two genera in themselves being centers for comparison with other genera of the family (FIG. 1).

Transition from *Comatricha,* with its globose to ovate or cylindrical sporangia, lacking a surface net, and having a partially persistent peridium, to *Lamproderma* with its globose to ovoid sporangia, its much-branched and anastomosing capillitium arising from a typically unbranched columella (*L. arcyrionema* is an exception) can conveniently be traced.

From *Stemonitis, Brefeldia* and *Amaurochaete* are divergent genera and possibly *Schenella. Brefeldia* Rost. (Versuch 8. 1873) has an aethalioid type of fructification consisting of confluent sporangia rising from a spongy barren base, columellae blending beneath and capillitial threads which arise from the columellae and unite with adjacent capillitial filaments by means of enlarged, many-chambered vesicles. The connection of the adjacent sporangia of *Stemonitis confluens* by means of the small circular discs on the lateral extensions of the capillitial filaments may be directly compared with the vesicles of *Brefeldia maxima* which unite similar elements.

Amaurochaete Rost. (Versuch 8. 1873) is likewise an aethalioid form with confluent sporangia, a fugacious peridium and an irregular capillitial complex of dark branches which sometimes forms an indistinct surface net, resembling the similar structure present in *Stemonitis*.

Schenella Macbride (Mycologia **3**: 39. 1911) is a genus of very doubtful affinities. For convenience it may be included with this group. It has an aethalioid fructification, a pale, fugacious, crust-

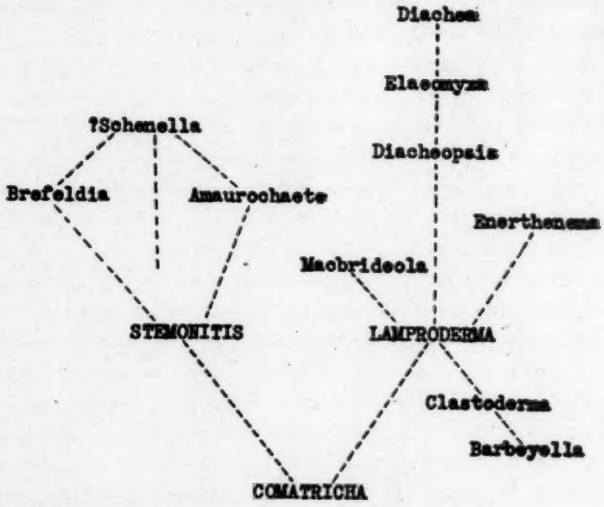


FIG. 1. Suggested relationships of the genera in the Stemonitaceae.

like peridium and abundant, dark brown capillitium, each column of which is made up of a number of twisted threads which form a cord covered in part at least by a sheath.

The following genera may be regarded as exhibiting characteristics which are indicative of relationships to *Lamproderma* with its typically globose, iridescent, stalked, or sessile sporangia with fairly persistent peridium, usually distinct columella from which the much-branched capillitium arises typically at the apex.

Diacheopsis Meylan (Bull. Soc. Vaud. Sci. Nat. **57**: 149. 1930) can best be compared with *Lamproderma insessum*, since both are sessile forms in which the columella is lacking. *Diacheop-*

sis seems to differ from typical Lamprodermas only in the absence of a columella. It is entirely possible that *L. insessum*, the only species of *Lamproderma* lacking a columella, should be transferred to *Diacheopsis*. This, however, would make the presence or absence of a columella the character on which the genera are distinguished. Because the presence or absence of a columella is a relatively unimportant difference on which to distinguish genera, it is suggested that the genus *Lamproderma* might appropriately be emended so as to include a form such as *Diacheopsis metallica*, which has all the other characteristics of *Lamproderma*. However, in the absence of sufficient material of either species for study, this is advanced merely as a suggestion.

Elaeomyxa Hagelstein (Mycologia **34**: 593. 1942) is a difficult genus to place. Hagelstein puts it in a family by itself on the basis of the presence of an oily or waxy substance in the stalk, columella, capillitium or sporangial wall. This segregation seems unwarranted. Its other characters: persistent peridium, capillitium of anastomosing dark threads, columella present or absent and dark spores, are in keeping with the concept of the family Stemonitaceae as here presented. The capillitium of *E. cerifera* is particularly suggestive of the capillitium of *Lamproderma scintillans*. The straight, rigid threads, sparsely branching and anastomosing, with pale tips, are striking in their resemblance to that species.

Diachea Fries (Syst. Orb. Veg. **1**: 143. 1825) is another genus whose relationships have been variously interpreted. In this treatment it is regarded as belonging to the Stemonitaceae, and closely related to *Lamproderma*, because of its iridescent peridium and its capillitium of delicate branching threads both of which are free from lime. The stipe and columella do contain lime but this does not seem sufficient reason for placing it in the calcareous families when lime is lacking in the capillitium and peridium, and when in other characteristics it so closely resembles *Lamproderma*. Therefore in this treatment it is placed as the end group of the family, for it may represent the connecting link between the limeless families and the lime-containing families, which would then, by implication, be derivative.

Enerthenema Bowman (Trans. Linn. Soc. **16**: 152. 1830) is a distinct genus which appears to be close to *Lamproderma*. It has

a well developed columella which extends the entire height of the sporangial cavity, ending in a flattened disc from which the flexuous capillitial filaments descend; the peridium tends to remain as a calyculus around the base of the sporangium.

Clastoderma Blytt (Bot. Zeitung **38**: 343. 1880), a unique genus, is characterized by sparsely branched capillitial threads which bear at their tips fragments of the peridium. It was brought out in the previous paper that *Lamproderma robustum* exhibits this same character to a less marked degree, but the relationship between the two genera is clear.

From *Clastoderma* to *Barbeyella* a transition in peridial characters may be observed. The peridium of *Barbeyella* dehisces in a few large irregular lobes; the dark capillitium radiates from the apex of the columella as in *Lamproderma* and is attached by the tips to the sporangial wall.

Macbrideola Gilbert (Univ. Iowa Stud. Nat. Hist. **16**: 155. 1934) is a genus of uncertain value. It is sporangiate, stipitate, has a fugacious or persistent peridium, a columella which may divide in some sporangia, but whether single or divided extends the entire height of the sporangial cavity and is attached to the peridium at its apex. In this latter respect it approaches *Enerthenema*. The rudimentary capillitium suggests its relationship with *Echinostelium*, which has been removed from the family. However, pending more collections of the two species (one according to Hagelstein) of *Macbrideola* it will be retained in the Stemonitaceae.

DEPARTMENT OF PLANT SCIENCE,
VASSAR COLLEGE,
POUGHKEEPSIE, NEW YORK

BIBLIOGRAPHY

1. **Albertini, J. B. de & L. D. de Schweinitz.** Conspectus fungorum. Lipsiae. 1805.
2. **Baker, Gladys E.** A comparative morphological study of the myxomycete fructification. Univ. Iowa Stud. Nat. Hist. **14** (8): 1–35. 1932.
3. **Berkeley, M. J.** Fungi. In Hooker, J. D., Flora Tasmaniae **2**: 241–282. 1859.
4. **Berkeley, M. J. & C. E. Broome.** Supplement to the enumeration of fungi of Ceylon. Jour. Linn. Soc. **15**: 82–86. 1876.
5. **Berlese, A. N.** In Saccardo Syll. Fung. **7**: 323–453. 1888.
6. **Cooke, M. C.** Handbook of British fungi. London. 1871.
7. ——. The Myxomycetes of Great Britain. London. 1877.

8. ——. The Myxomycetes of the United States. Ann. Lyc. Nat. Hist. N. Y. **11**: 378–409. 1877.
9. **Crowder, William.** Marvels of Mycetozoa. Nat. Geog. Mag. **49**: 421–443. 1926.
10. **Ellis, J. B. & B. M. Everhart.** New species of fungi from Washington Territory. Bull. Washburn Lab. Nat. Hist. **1**: 3–6. 1884.
11. **Fries, Elias.** Systema mycologicum **3**: 67–198. 1829.
12. **Gilbert, Henry C.** Three new species of Myxomycetes. Univ. Iowa Stud. Nat. Hist. **16**: 153–159. 1934.
13. **Graff, P. W.** Contributions to our knowledge of western Montana fungi. I. Myxomycetes. Mycologia **20**: 101–113. 1928.
14. **Hagelstein, Robert.** An Adirondack myxomycete. Mycologia **27**: 86–89. 1935.
15. ——. A new genus of the Mycetozoa. Mycologia **34**: 593–594. 1942.
16. ——. Mycetozoa of North America. New York. 1944.
17. **Hattori, H.** Myxomycetes of Nasu District. 1935.
18. **Hennings, P.** Myxomycetes. Hedwigia **35**: 207–209. 1896.
19. **Leveille, J. H.** Fungi. In Triana, J. and J. E. Planchon. Prodromus florae Novo-Granatensis. Ann. Sci. Nat. IV. **20**: 289–290. 1863.
20. **Link, H. F.** Handbuch zur Erkennung der Nutzbarsten und am haufigsten vorkommenden Gewachse **3**: 405–422. 1833.
21. **Lister, A.** Notes on Mycetozoa. Jour. Bot. **29**: 257–268. 1891.
22. ——. A Monograph of the Mycetozoa. London. 1894.
23. ——. A Monograph of the Mycetozoa. Second edition revised by Gulielma Lister. London. 1911.
24. ——. A Monograph of the Mycetozoa. Third edition revised by Gulielma Lister. London. 1925.
25. **Lister, G.** Mycetozoa found during the fungus foray in the Forres District, Sept. 12–20, 1912, with the description of a new species. Trans. Brit. Myc. Soc. **4**: 38–44. 1912.
26. ——. Two new varieties of *Lamproderma*. Jour. Bot. **57**: 25–27. 1919.
27. ——. New or rare Mycetozoa. Jour. Bot. **59**: 89–93. 1921.
28. ——. *Lamproderma columbinum* Rost. and its varieties. Trans. Brit. Myc. Soc. **9**: 32–34. 1923.
29. **Macbride, T. H.** Nicaraguan Myxomycetes. Bull. Lab. Nat. Hist. Iowa **2**: 377–383. 1893.
30. ——. The North American Slime-Moulds. New York. 1899.
31. ——. The North American Slime-Moulds. New and revised edition. New York. 1922.
32. —— **& G. W. Martin.** The Myxomycetes. New York. 1934.
33. **Martin, G. W.** Systematic position of the slime moulds and its bearing on the classification of the fungi. Bot. Gaz. **93**: 421–435. 1932.
34. ——. The Myxomycetes. Bot. Rev. **6**: 356–388. 1940.
35. **Massee, George.** A Monograph of the Myxogastres. London. 1892.

36. **Meylan, Ch.** Myxomycetes du Jura. Bull. Soc. Vaud. Sc. Nat. **46**: 49–57. 1910.
37. ——. Myxomycetes du jura. Bull. Soc. Geneve **II. 6**: 86–90. 1914.
38. ——. Nouvelles contributions a l'etude des Myxomycetes du Jura. Bull. Soc. Vaud. Sc. Nat. **51**: 259–269. 1917.
39. ——. Myxomycetes nouveaux. Bull. Soc. Vaud. Sc. Nat. **52**: 95–97. 1918.
40. ——. Notes sur quelques especes de Myxomycetes. Bull. Soc. Vaud. Sc. Nat. **52**: 447–450. 1919.
41. ——. Contribution a la connaissance des Myxomycetes de la Suisse. Bull. Soc. Vaud. Sc. Nat. **53**: 451–463. 1921.
42. ——. Recherches sur les Myxomycetes du Jura en 1921–22–23. Bull. Soc. Vaud. Sc. Nat. **55**: 237–244. 1924.
43. ——. Note sur divers Myxomycetes du Jura et des Alpes. Bull. Soc. Vaud. Sc. Nat. **56**: 65–74. 1925.
44. ——. Recherches sur les Myxomycetes du Jura en 1925–26. Bull. Soc. Vaud. Sc. Nat. **56**: 319–328. 1927.
45. ——. Recherches sur les Myxomycetes en 1927–28. Bull. Soc. Vaud. Sc. Nat. **57**: 39–47. 1929.
46. ——. Note sur un nouveau genre de Myxomycetes. Bull. Soc. Vaud. Sc. Nat. **57**: 147–149. 1930.
47. ——. Les especes nivales du genre *Lamproderma*. Bull. Soc. Vaud. Sc. Nat. **57**: 359–373. 1932.
48. **Meylan, Ch.** Recherches sur les Myxomycetes du Jura 1930–31–32. Bull. Soc. Vaud. Sc. Nat. **58**: 81–90. 1933.
49. ——. Recherches sur les Myxomycetes suisses en 1933–34. Bull. Soc. Vaud. Sc. Nat. **58**: 319–320. 1935.
50. ——. Myxomycetes japonais. Bull. Soc. Vaud. Sc. Nat. **58**: 321–324. 1935.
51. **Morgan, A. P.** Myxomycetes of the Miami Valley, Ohio. Jour. Cin. Soc. Nat. Hist. **16**: 127–156. 1894.
52. ——. Myxomycetes of the Miami Valley, Ohio. Jour. Cin. Soc. Nat. Hist. **19**: 147–166. 1900.
53. **Persoon, C. H.** Observationes mycologicae. Usteri's Ann. Bot. **15**: 1–39. 1795.
54. ——. Synopsis methodica fungorum. 1801.
55. **Raciborski, M.** Ueber einige neue myxomyceten Polens. Hedwigia **28**: 115–124. 1889.
56. **Raunkiaer, C.** Myxomycetes Daniae. Bot. Tidskr. **17**: 20–105. 1888. Description in English of new species described. id. **17**: 106–110. 1889.
57. **Rostafinski, J. T.** Versuch eines Systems der Mycetozoen. Strassburg. 1873.
58. ——. Sluzowce (Mycetozoa): Monografia. Pamiet. Towarz. Nauk Paryzu **5** (4): 1–215. 1874.
59. ——. Dodatek i do monografii sluzowcow. Pamiet. Towarz. Nauk Paryzu **8** (4): 1–45. 1876.
60. **Saccardo, P. A.** Syll. Fung. **11**: 462–471. 1895.
61. —— **& H. Sydow.** Syll. Fung. **14**: 831–840. 1899.
62. —— **& A. Trotter.** Syll. Fung. **22**: 792–816. 1913.
63. **Torrend, C.** Flore des Myxomycetes. Broteria **6–8**: 1907–1909.

TWO NEW SPECIES OF MONOBLEPHARELLA [1]

MARTHA E. SPRINGER

(WITH 51 FIGURES)

Soils collected from localities widely distributed in the Western Hemisphere have yielded a number of isolates of fungi referable to the genus *Monoblepharella*. A study has been made of the development and morphology of the ten isolates producing sex organs, and will be reported in a later paper. Cultures of the original isolates of *Monoblepharella Taylori* Sparrow and *M. mexicana* Shanor were furnished by the authors. It was possible, therefore, to make careful comparisons with the only two members of the genus which have already been described. This study has resulted in the addition to the genus of two new species.

Monoblepharella elongata sp. nov.

Mycelium well developed; hyphae sparingly branched, the branches usually arising at right angles to the main axes, 1.5–4 μ in diameter, the stouter basal portions up to 7 μ; vacuolization reticulate or scalariform; hyphae usually with many irregular swellings. Sporangia narrowly cylindrical or siliquiform, 40–120 μ in length by 5–13 μ in diameter at the widest point, tapering to 2–4 μ at the base, terminal, or after sympodial branching of the hyphae, appearing lateral, usually with one or occasionally several lateral papillate outgrowths near the base. Zoospores emerging through a pore at the apex of the sporangium and through the apices of outgrowths; zoospore with an anterior group of small refractive globules, ovoid or subcylindrical, 6.5–10.5 μ long by 4–6 μ wide, the single posterior flagellum up to 26 μ long. Oogonium at first terminal or, after sympodial branching of the supporting hypha, appearing lateral,

[1] Contribution from the Botany Department, University of Michigan, no. 744.

This paper is part of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

narrowly obpyriform, 17–35 μ long by 7–12 μ in diameter at the widest point, with rounded apex and a cylindrical base 2–4 μ in diameter, the contents at maturity forming one to three or rarely up to eight eggs containing numerous large refractive globules. Antheridium either terminal on a branch subtending the oogonium and cylindrical, 20–45 μ long by 3–5 μ wide, or hypogynous and geniculate, consisting of a cylindrical section of the suboogonial hypha 2–19 μ long and a beaklike lateral outgrowth 10–35 μ long by 3–5 μ wide. Antherozoids four to seven, emerging through a pore at the apex of the antheridium, strongly amoeboid, ovoid when swimming, 5–6 μ long by 3–4 μ wide, with an anterior group of small refractive globules, posteriorly uniflagellate, the flagellum up to 20 μ in length. Zygote spherical or broadly ovoid, 9–13 μ long by 8–10 μ wide, posteriorly uniflagellate, free swimming or during rest periods strongly amoeboid, containing large refractive globules. Oospore formed free in the water, or occasionally retained within or at the orifice of the oogonium, 9–12 μ in diameter, with a light brown, smooth wall up to 1 μ in thickness, contents bearing globules, upon germination forming a mycelium.

In soil, isolated on hemp seed bait. Soil collected by C. D. LaRue from ditch behind rubber factory, Las Palmas, State of Chiapas, Mexico, February 6, 1941 (type of species). Soil collected by M. E. Springer from marble quarry, Columbia, Tuolumne County, California, March 28, 1942.

Mycelium amplum, hyphis ramosis, tenuibus 1.5–7 μ diametro, reticulate vel scalariforme vacuolatis, plerumque multis irregularibus tumoribus. Sporangia anguste cylindrica vel siliquiformia, 40–120 μ longa, 5–13 μ crassa, basi angustata 2–4 μ crassa, plerumque uno aut rare compluribus papilliformis protrusionibus ad basum; zoosporis ovoideis vel subcylindricis, 6.5–10.5 μ longis, 4–6 μ crassis, uno postico flagello usque ad 26 μ longo. Oogonium hypham terminans, anguste obpyriforme, 17–35 μ longum, 2–4 μ crassum, apice rotundato, basi anguste cylindrico 2–4 μ diametro, ovis 1–3 rare ad 8 cum globulis magnis refractivis. Antheridium aut hypham oogonio subnatam terminans, anguste cylindricum, 20–45 μ longum, 3–5 μ diametro, aut hypogynum, segmenti hyphae suboogonialis 2–19 μ longi et protrusionis rostriformae lateralis 10–35 μ longi, 3–5 μ crassi; antherozoideis 4–7 valde amoeboideis, ovoideis si natantibus, 5–6 μ longis, 3–4 μ crassis, uno postico flagello ad 20 μ longo. Ova inseminata sphaerica vel late ovoidea, 9–13 μ longa, 8–10 μ crassa, uno postico flagello, natantia aut, dum requiescent, valde amoeboidea, globulis magnis refractivis inclusis. Oospora in aqua libere formata sphaerica, 9–12 μ diametro, membrana laevia pallide brunnea, ad 1 μ crassa, globulis inclusis, germinata mycelium formans.

In humo per *Cannabis* semen illecebram culta. Humum legit C. D. LaRue e fossa, Las Palmas, Civitas Chiapas, Mexico, Feb. 6, 1944 (specimen typicum).

Monoblepharella elongata appears to be most closely related to *M. Taylori* Sparrow. The order of development and the arrangement of the organs is the same. The most conspicuous difference between the two species is that which suggested the specific name *elongata* for the new species—a pronounced elongation in *M. elongata* of all of the organs, sporangia, as well as antheridia and oogonia, without a corresponding increase in diameter.

Many of the stages in the morphology and development of *Monoblepharella elongata* are similar to those which have already been described for *Monoblepharis* (Sparrow, **1933, 1943**) and for other species of *Monoblepharella* (Sparrow, **1940**; Shanor, **1942**). The mycelium, like that of other species of *Monoblepharella,* is sufficiently distinctive to identify it as belonging to the genus even in the vegetative condition. It grows very slowly and is so delicate that it is distinguishable even from other members of the *Monoblepharidales,* all of which possess the same type of reticulate or scalariform vacuolization. Globose or irregular swellings (FIG. 9) occur in great abundance on the hyphae of *M. elongata.* Such nodules are found to some extent on the hyphae of all other species (Sparrow, **1940**; Shanor, **1942**), but not in such large numbers. They are considered to be normal structures, probably acting as reservoirs of protoplasm for subsequent vegetative growth.

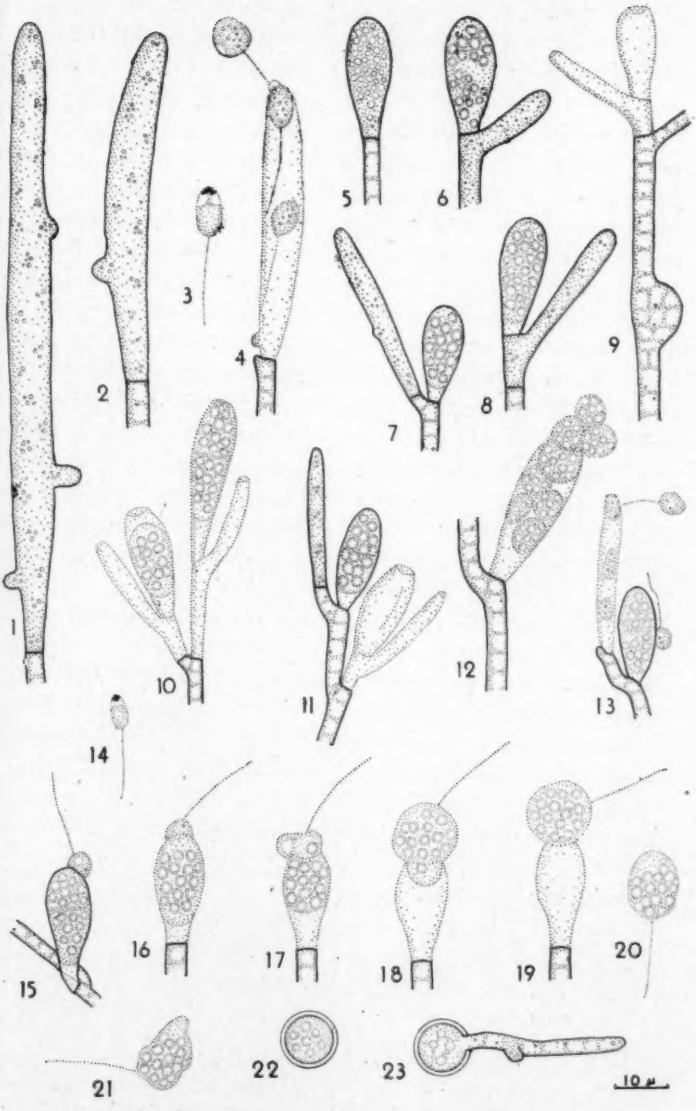
In undisturbed cultures only a few reproductive organs will develop at any one time. Although both nonsexual and sexual organs are occasionally found simultaneously, more typically either the one or the other is formed under given conditions. It has been found that production of sporangia is stimulated by the sudden starving of the mycelium by placing it in fresh water at a temperature preferably between 21 and 31° C. Sex organs are formed in abundance only when the mycelium is left undisturbed for several days at constant temperatures ranging from 26–32° C., with the optimum in this species from 26–27° C.

By reason of their refractive appearance and large size as compared with the delicate mycelium, the sporangia of *Monoblepharella elongata* are very conspicuous. Although there is much overlapping in the measurements of the sporangia of this and of other species, the average length in *M. elongata,* 75 μ, is much greater while the average width, 8.6 μ, is somewhat less. The mean ratio

of length to diameter is more than one and one-half times that of *M. Taylori*. The presence of lateral papilla-like projections has been mentioned for *M. Taylori* and *M. mexicana*, but in both of these species they occur only rarely. The sporangia of *M. elongata* (FIGS. 1, 2, 4) typically bear one or more of these lateral projections which are used as additional exit tubes, although the majority of the zoospores escape through the apex. The zoospores in the manner of their escape and appearance (FIG. 3) are indistinguishable from those described for other species.

Both oogonia and antheridia are at once distinguished from those of congeneric forms by their long narrow shape and the frequency with which more than one egg is formed in the oogonium. The method of development and the resulting arrangement of sex organs are like those described in *Monoblepharella Taylori* (Sparrow, **1940**). The oogonial rudiment develops terminally on a hypha (FIG. 5). Small oil droplets coalesce to form the large globules (FIG. 6) characteristic of the mature egg. Although at first they fill the oogonium, later the globules are found only in the central portion, leaving an area of clear protoplasm at the tip and at the base. At maturity the egg rounds up and withdraws from the base of the oogonium. If more than one egg is to be formed in the oogonium, this is indicated by a characteristic grouping of the oil globules before the actual delimitation is noticeable. In about one-half of the oogonia more than one egg is produced. The number is usually from one to three, although eight were observed in a single oogonium (FIG. 12). This is in striking contrast to the other species where it is only rarely that an oogonium contains more than one egg.

Antheridial growth is initiated by the development of a short lateral branch arising immediately beneath the oogonial cross wall (FIG. 6). The antheridium may be terminal (FIGS. 7, 11) or hypogynous (FIGS. 8–11), with both types sometimes found even on the same branch (FIG. 11). The antheridia average about eight times as long as wide, while in *M. Taylori* this ratio is only about three to one. The emergence of the antherozoids (FIG. 13) is similar to that of the zoospores. While emerging the antherozoid is round or irregular at first, but when swimming it resembles a small zoospore (FIG. 14). When it is moving about on the surface



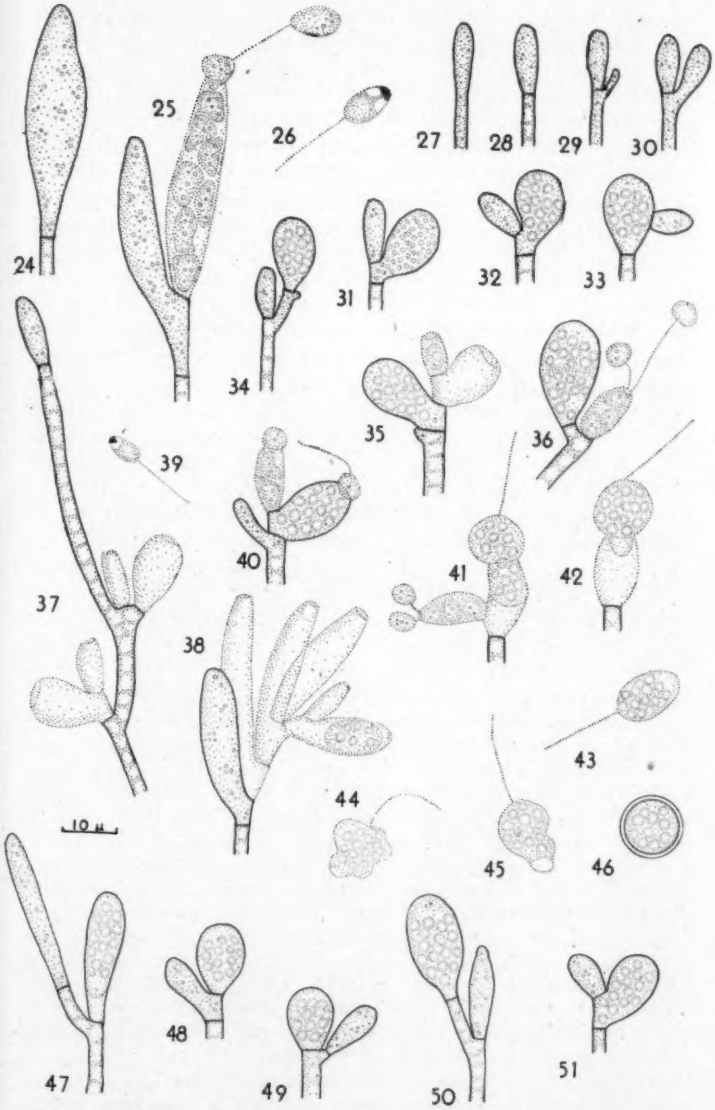
FIGS. 1–23. *Monoblepharella elongata.*

of an oogonium (FIG. 15), it is strongly amoeboid, its flagellum remaining passive, or waving weakly about in the medium. Fertilization and the emergence of the zygote (FIGS. 16–19) occur precisely as in *M. Taylori*. Indeed, save for the difference in the shape of the sex organs, the figures of this process as occurring in *M. Taylori* (Sparrow, **1940**) could be used for any of the species studied. The zygote (FIG. 20) too is characteristic of the genus. As it swims with a rolling, halting and rocking movement, it progresses so slowly that it is frequently possible to see the moving posterior flagellum. The flagellum may be visible throughout the entire period of fertilization, emergence, and the early swimming stages, thus giving definite evidence that it is, as Sparrow contended, the flagellum of the antherozoid which is used for the propulsion of the zygote. As the zygote rolls and turns the globules are easily distinguishable and seem to form a compact peripheral layer lining all except the broadly conical anterior end. Motility may continue for a period of at least half an hour, during which time it frequently stops to rest or to undergo strong amoeboid crawling (FIG. 21). Finally it encysts and becomes surrounded by a smooth thickened wall (FIG. 22).

Germination of the oospores may be obtained by adding fresh water and bait to an old culture or to the dried spores. It is not known how long the spores will remain viable, but growth of this species was obtained from dried soil from the original sample three years after collection. Germination is by a hypha which protrudes through a pore formed in the wall of the oospore (FIG. 23). This primary vegetative element almost immediately assumes the vacuolate condition typical of the mature mycelium.

Monoblepharella Laruei sp. nov.

Mycelium well developed; hyphae sparingly branched, the branches usually arising at right angles to the main axes, delicate, 1.5–4 μ in diameter, the stouter basal portions up to 7 μ; vacuolization reticulate or scalariform; hyphae with occasional swellings. Sporangia cylindrical or siliquiform, 28–82 μ long by 7–15 μ in diameter at the widest point, occurring at the tips of hyphae, or several often developed in basipetal succession, later sporangia sometimes geniculate. Zoospores emerging through a pore at the apex of the sporangium, ovoid or somewhat cylindrical, 7–9 μ long by



FIGS. 24–26, *Monoblepharella Larucei;* 47, *M. elongata;* 48 and 49, *M. Taylori;* 50, *M. mexicana;* 51, *M. Larucei.*

5–5.5 μ in diameter, with an anterior group of small refractive globules, posteriorly uniflagellate, the flagellum up to 27 μ in length. Oogonium terminal on a branch subtending the anteridium, or formed by a swelling of the hypha below the antheridium, after sympodial branching of the hypha appearing lateral, obpyriform, 11–20 μ long by 7–12 μ in diameter at the widest point, with rounded apex, and, if terminal, with narrowly cylindrical base tapering to 2–4 μ, the contents at maturity forming one or rarely up to three eggs bearing numerous large refractive globules. Antheridium always formed terminally on the hypha, later often epigynous, 8–19 μ long by 4–7 μ in diameter at the widest point. Antherozoids two to five, emerging through a pore at the apex of the antheridium, strongly amoeboid, ovoid when swimming, 4–5 μ long by 3–3.5 μ wide, with an anterior group of small refractive globules, posteriorly uniflagellate, the flagellum up to 19 μ in length. Zygote spherical or broadly ovoid, 10–13 μ long by 8–10 μ wide, posteriorly uniflagellate, free swimming, amoeboid during rest periods, the contents bearing numerous large refractive globules. Oospore formed free in the water, spherical, 9–13 μ in diameter, with a light brown, smooth wall up to 1 μ in thickness, contents bearing globules, germination not observed.

In soil, isolated on hemp seed bait. Soil collected by C. C. LaRue: stream bank mud, Cooper Landing, near Bluefields, Nicaragua, December 28, 1940 (type).

Mycelium amplum, hyphis ramosis, tenuibus 1.5–7 μ diametro, reticulate vel scalariforme vacuolatis, spatio interiecto tumoribus. Sporangia cylindrica vel siliquiformia 28–82 μ longa, 7–15 μ crassa, terminalia vel saepe plura basipetaliter producta; zoosporis ovoideis vel cylindricis 7–9 μ longis, 5–5.5 μ crassis, uno postico flagello usque ad 27 μ longo. Oogonium hypham antheridio subtenentem terminans aut e hypha sub antheridio tumescente formatum obpyriforme, apice rotundata 11–20 μ longum, 7–12 μ crassum, et si terminale basi anguste cylindrico 2–4 μ diametro; ovis singulis rare ad 3 cum globulis magnis refractivis. Antheridium semper hypham terminans, postea aliquando epigynum 8–19 μ longum, 4–7 μ crassum; antherozoideis 2–5 valde amoeboideis, ovoideis si natantibus, 4–5 μ longis, 3–3.5 μ crassis, uno postico flagello ad 19 μ longo. Ova inseminata sphaerica vel late ovoidea 10–13 μ longa, 8–10 μ crassa, natantia, dum requiescent amoeboidea, magnis refractivis globulis inclusis. Oospora in aqua libere formata, sphaerica, 9–13 μ diametro, membrana pallide brunnea laevia ad 1 μ crassa, magnis refractivis globulis inclusis, germinatione ignota.

In humo per Cannabis semen illecebram culta. Humum legit C. D. LaRue e luto in rivi ripa, Cooper Landing, ad Bluefields, Nicaragua, December 28, 1940 (specimen typicum).

This fungus is dedicated to Dr. C. D. LaRue of the University of Michigan, who furnished a large number of the soil samples from which were obtained isolates of *Monoblepharella,* including the type isolates of *M. Laruei and M. elongata.*

The mycelium of *Monoblepharella Laruei* is typical for the genus. Some nodules are present although not in such great quantity as on hyphae of *M. elongata.* Sporangia are produced in great abundance when the mycelium is put into fresh water between temperatures of 21–31° C. They develop terminally on the hypha (FIG. 24) and in shape and size closely resemble those of *M. Taylori.* Secondary sporangia (FIGS. 25, 38) however, are formed in basipetal succession rather than in the sympodial manner characteristic of the other species. This type of sporangial formation has been found to occur in a few instances but not typically in cultures of *M. mexicana.* The swelling below the cross wall of the primary sporangium develops directly into a geniculate sporangium, which is then delimited basically from its attendant hypha by a cross wall. Sporangia may continue to form in this manner. Usually as each sporangium develops it comes to lie with its long axis in line with that of the hypha. The first formed sporangium now appears to be a lateral outgrowth from the mature secondary organ. Sporangial discharge and the zoospores (FIG. 26) are like those of other species.

Development of the sex organs proceeds in just the reverse order from that of *Monoblepharella elongata* and *M. Taylori,* starting with the formation of the antheridium, as in *M. mexicana.* The antheridium is formed by the swelling of a hyphal tip (FIG. 27) and the laying down of a cross wall (FIG. 28). The mature antheridium has the smallest average length (11 μ) of any of the isolates studied. The lateral outgrowth (FIG. 29) which soon forms beneath the antheridial cross wall may develop into a short branch, and produce a terminal oogonium (FIG. 34), or it may expand to form a subantheridial oogonium (FIGS. 30, 31). This method of oogonial development corresponds closely to that of the hypogynous antheridia of *M. Taylori* and *M. elongata.* When the oogonium is first cut off, the antheridium, although now epigynous, lies in a direct line with the long axis of the hypha. Frequently, however, the oogonium rather than the antheridium comes

to lie in this position, and the antheridium then appears lateral (FIGS. 32, 33). Occasionally a second female gametangium may be cut off immediately below the first (FIG. 35). By later sympodial branching of the hypha, the sex organs may assume a lateral position (FIG. 37).

The sequence of stages in the formation of the egg, involving the coalescence of oil droplets to form large globules, progresses as in other species. Rarely more than one egg may be formed in an oogonium (FIG. 36). Fully mature oogonia are obpyriform or geniculate, depending upon whether they are terminal or intercalary.

Sex organs have been developed abundantly in cultures kept at constant temperatures of between 26 and 32° C. If the culture is disturbed or removed from the oven, sporangial development will commence. Occasionally in such conditions sporangia will be formed basipetally beneath sex organs (FIG. 38).

Because of the small size of the antheridium, frequently there are only two antherozoids produced in each. The largest number observed in a single antheridium was five. The emergence of the antherozoids (FIGS. 36, 40, 41), their appearance when swimming (FIG. 39), fertilization (FIG. 40) and the emergence and the swarming of the zygote (FIGS. 41–45) are like these stages in other members of the genus. Large numbers of resting spores may be found in an old culture, but their germination has not as yet been observed.

Resemblances have been pointed out between *Monoblepharella Laruei* and *M. mexicana* and also between *M. elongata* and *M. Taylori*. Distinctive characteristics of the four species are emphasized by a comparison of drawings of typical pairs of sex organs. The organs of *Monoblepharella elongata* (FIG. 47) are much longer and comparatively narrower than the more rounded organs of *M. Taylori* (FIGS. 48, 49). In both of these fungi the oogonium is produced before its attendant antheridium, and the antheridium may be either terminal or hypogynous. In *Monoblepharella mexicana* (FIG. 50) and *M. Laruei* (FIG. 51) the oogonium develops after its attendant antheridium. In *M. mexicana* both organs are produced terminally on the hyphae, while in *M. Laruei* the oogonium is frequently epigynous.

SUMMARY

Two new species of *Monoblepharella* have been recovered from soil samples, *Monoblepharella elongata* from Mexico and California and *Monoblepharella Laruei* from Nicaragua. *Monoblepharella elongata* in the development and arrangement of its organs resembles *M. Taylori* Sparrow. It differs from Sparrow's species in the large number of nodules on the mycelium, the presence typically of lateral projections on the zoosporangia, the frequent formation of several eggs in the oogonia, and, most conspicuously, in the greater length and slenderness of its reproductive organs. *Monoblepharella Laruei* is the only known species of *Monoblepharella* in which epigynous antheridia may occur. This, the small size of the antheridia, and the formation of secondary zoosporangia almost exclusively in basipetal fashion are the chief characteristics which separate it from the closely related *M. mexicana* Shanor.

ACKNOWLEDGMENTS

The writer wishes to express her appreciation to Dr. C. D. LaRue for the use of the soils from which the new species were isolated, to Dr. Leland Shanor for the culture of *Monoblepharella mexicana,* and to Dr. F. K. Sparrow for the culture of *M. Taylori.* She is especially indebted to Dr. Sparrow for his helpful suggestions during the investigation and the preparation of this paper.

DEPARTMENT OF BOTANY AND BACTERIOLOGY,
INDIANA UNIVERSITY,
BLOOMINGTON, INDIANA

LITERATURE CITED

Shanor, L. 1942. A new *Monoblepharella* from Mexico. Mycologia **34**: 241–247. *fig. 1–20.*

Sparrow, F. K., Jr. 1933. The Monoblepharidales. Ann. Bot. **47**: 517–542. *pl. 20.*

——. 1940. Phycomycetes recovered from soil samples collected by W. R. Taylor on the Allan Hancock 1938 Expedition. Allan Hancock Pacific Expeditions, Publ. Univ. So. Calif. **3**: 101–112. *pl. 16, 17.*

——. 1943. Aquatic Phycomycetes (exclusive of the Saprolegniaceae and *Pythium*). xix–785 pp. *69 fig.* University of Michigan Press, Ann Arbor.

EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida and are approximately × 758 as here reproduced.

FIGS. 1–23, *Monoblepharella elongata.* 1, large zoosporangium with three lateral projections; 2, zoosporangium of average size; 3, zoospore; 4, zoosporangium showing late stage in emergence of zoospores; 5, young oogonium; 6, older oogonium and antheridial rudiment; 7, sex organs, with antheridium terminal; 8, sex organs, with antheridium hypogynous; 9, empty sex organs on a portion of mycelium bearing a nodule; 10, branch with two closely adjacent pairs of sex organs; antheridia hypogynous in both; 11, two pairs of sex organs, showing both terminal and hypogynous antheridia on the same branch; 12, large oogonium with eight eggs; 13, stage in emergence of antherozoids; 14, antherozoid; 15, antherozoid on tip of oogonium: 16–19, fertilization and stages in the emergence of the zygote; 20, typical motile zygote; 21, zygote with shape assumed during periods of amoeboid movement; 22, oospore; 23, germination of oospore.

FIGS. 24–46, *Monoblepharella Laruei.* 24, typical sporangium; 25, stage in discharge of zoospores from a terminal sporangium which is subtended by a basipetally formed secondary sporangium; 26, zoospore; 27–33, stages in development of sex organs, showing terminal development of antheridium, and subsequent development of oogonium so that the antheridium appears to be epigynous; 34, hyphal tip on which both the antheridium and the oogonium were formed terminally; 35, antheridium subtended by two oogonia produced in basipetal succession; 36, large oogonium with the two eggs; antheridium from which antherozoids are emerging; 37, portion of hypha showing sequence of sex organs; 38, hyphal tip showing basipetal development of four sporangia beneath an oogonium and epigynous antheridium; 39, antherozoid; 40, antherozoid about ready to fertilize egg; 41, 42, stages in emergence of zygotes; 43, typical motile zygote; 44, 45, shapes assumed by zygote during periods of amoeboid movement; 46, oospore.

FIGS. 47–51, typical sex organs of four species of *Monoblepharella.* 47, *M. elongata;* 48, 49, *M. Taylori;* 50, *M. mexicana;* 51, *M. Laruei.*

A CYTOLOGIC STUDY OF SEVERAL SMUT FUNGI

ELISA HIRSCHHORN [1]

(WITH 3 FIGURES)

In spite of the fact that the Ustilaginales, from the biologic and economic point of view, are one of the most important groups of parasitic fungi in the world, not much has been done to explain the biological phenomena associated with the behavior of their nuclei. Most of the work has been done in taxonomy and in the life history and physiology. Genetic knowledge of smuts, especially those which parasitize cereals, such as *U. Zeae, U. levis, U. Hordei,* etc., shows that the chlamydospores possess diploid nuclei, which migrate to the young promycelia when the chlamydospores germinate, and that the first and second nuclear divisions take place in the promycelium, resulting in the formation of four cells, each containing one haploid nucleus (**4**, **5**, **6**, **13**, **14**, **17**, **18**). According to Hanna (**8**), Christensen (**2**) and others (**11**, **16**), reduction may take place in the first, second, or later nuclear divisions. These genetic studies have been confirmed by cytologic investigations. In fact, Wang, D. T. (**18**) showed cytologically, in *U. Avenae, U. levis, U. Hordei, U. nuda, U. violacea, U. longissima,* etc., that reduction may take place in the first or second nuclear division. These species have two pairs of chromosomes, the first nuclear division being reductional and the second equational. The first division may take place inside the spore at the moment when germination begins, the reduced nuclei migrating to the young promycelium,

[1] Assistant in Plant Pathology, The State College of Washington, Pullman, Washington.

Acknowledgment is due to Dr. Hannah Aase, Dr. George W. Fischer, Dr. C. S. Holton, and Dr. E. J. Anderson for aid in interpreting the microscopic preparations. Thanks are also due Dr. Holton and Dr. Anderson for the English correction of the manuscript.

Published as Scientific Paper No. 615, College of Agriculture, Agricultural Experiment Station, State College of Washington.

or in other cases all division may take place in the promycelium. Recently Wang, C. S. (**17**) showed that *U. Crameri* also has two pairs of chromosomes and that meiotic division may take place in the first, second, or third nuclear division. These caryologic studies are the physical basis which explains genetic segregation observed in the species of *Ustilago* mentioned above, and the formation of many physiologic races, but they do not explain why some species, for example *U. Zeae,* are made up of an indefinite number of physiologic forms, more than is possible to expect by segregation and recombination. Christensen (**2**) has studied a large number of different sexual types of segregation in *U. Zeae.* He believed, like Stakman (**16**), that existence of so many different sexual groups and so many different physiologic forms in this species may be due, in part, to late reductional characters. Nuclear behavior in very few other species has been studied. In *U. longissima, U. Zeae,* and *Tilletia Tritici* the haploid number of chromosomes is also two (**18**). It is evident that more study of the nuclear process in smut fungi is needed.

As a part of a more extensive and detailed cytologic work, some preliminary observations on nuclear behavior of *U. Williamsii, U. Spegazzinii* var. *agrestis, U. halophila* and *Sorosporium consanguineum* are presented here. Different stages in nuclear division in the promycelium, sporidial and hyphal formations, and some abnormalities in these will be described. The first three species listed are stem smuts and the fourth a flower smut. *U. Spegazzinii* var. *agrestis* and *U. Williamsii* have a similar peculiarity of morphology: both have prolongations of the epispore, although these prolongations are different in nature. In the second species they are hyaline, while in the first they are the same as the epispore. Probably this may involve some relationship. In some years *U. Spegazzinii* is of economic importance, being endemic in the northwestern part of the United States and in La Plata, Argentina.

MATERIAL AND METHODS

The material used in the present study was kindly supplied by Dr. G. W. Fischer. It was collected in Montana, Washington, and Idaho and consisted of *Ustilago Williamsii* on *Stipa Richardsoni,* from Butte, Mont., 1941; *U. Spegazzinii* var. *agrestis,* on *Agro-*

pyron Smithii, Pullman, Wash., 1943; *U. halophila,* on *Distichlis stricta,* Blue Lake, Idaho, 1940: *Sorosporium consanguineum,* on *Aristida longis,* Whitebird, Idaho, 1941.

The spores were germinated in an aqueous solution containing 2 per cent dextrose, 1 per cent maltose, and 0.5 per cent peptone extract. The slides were covered with a very thin film of egg albumin. After spreading a drop of nutrient solution on the slide, the spores were dusted on by means of a thin brush. The slides were then placed in a moist petri dish, inside an incubator, at 22–25° C. When the desired stages were reached, the spores on the slides were killed and fixed with Fleming's weak solution by putting several drops of the solution on the slide and leaving it 20–25 minutes, after which period the liquid was removed with filter paper. When dry, the material was bleached by placing the slides in a 10 per cent solution of hydrogen peroxide for 20 minutes, after which they were washed in distilled water 3–5 minutes. The slides were then placed in 4 per cent iron alum 4–6 hours and washed in distilled water 2–3 minutes. Staining was done in an aqueous 0.5 per cent solution of haematoxylin for 18–24 hours, following which the slides were washed in running water 10 minutes and then 10 minutes in distilled water, changing two or three times, as suggested by Rawlins (**15**). Dehydration was begun with 15 per cent alcohol, allowing 2 minutes in each grade. After 95 per cent alcohol the material was destained in acid alcohol for about 10 seconds, and then placed again in 95 per cent and finally in 100 per cent alcohol. Clearing was done in clove oil which was washed out with two changes of xylol. The stained preparations were mounted in balsam. Other fixatives used included Craf's, Bouin's and a solution made up of 3 parts of absolute alcohol and one part of glacial acetic acid, and other stains used included Crystal Violet and Triple Stain, but Fleming's weak solution and haematoxylin gave the best results.

RESULTS

USTILAGO WILLIAMSII (Grif.) Lavr.

Spores dark olivaceous-brown, globose to subglobose, 7–10 μ in diameter; epispore smooth and deeply cracked, bearing two bipolar

appendages. Germination begins after 12 hours storage under favorable conditions. Behind the appendages a tube emerges which in a few hours reaches complete development, forming a 4–8 celled promycelium, which soon begins to form branches, and in many cases these branches developed secondary branches. In the culture medium used in this study such sporidia did not appear (**10**).

Nuclei behavior. The nuclear behavior as observed in various stages of development in this species is illustrated in figure 1. The mature chlamydospore contains one nucleus which apparently is diploid (FIG. 1: 1). This is similar to the nuclear conditions observed in other smut fungi (**4**, **13**, **14**, **17**, **18**). Apparently the first nuclear division sometimes occurs in the spore but in most cases in the very young promycelium. This nucleus migrates to the promycelium as soon as a short tube appears, usually without previous division (FIG. 1: *2*, 4). Division stages inside the spores were not observed. The only suggestion that such division occurs is the presence of nuclei inside the spore after a long promycelium has developed and the occurrence of bipolar germination (FIGS. 1: *17*, *22*). When bipolar germination does occur, nuclear division must occur inside the spore and when the promycelium emerges, one nucleus migrates into the larger promycelium while the other seems to remain for a longer time, apparently undergoing a new division inside the spore (FIG. 1: *17*, *22*). It was not possible to observe many prophase stages. In the few cases observed, the nuclear structure was as illustrated in figure 1: *2* and 3, which appears to be more a resting stage. There is a thin reticulum, a nucleolus and a very thin nuclear membrane. Soon after the nucleus reaches the young promycelium, some times at the base and sometimes at the apex, it is possible to observe four deeply stained bodies which have the appearance of chromosomes in the metaphase (FIG. 1: 4). They soon separate and only two chromosomes are observable in each group in the anaphase (FIG. 1: 5). The second division follows without a resting stage, or at least a resting stage was not observed. Figure 1: 6 and *7* show the second anaphasic stage of such a division with two chromosomes in each one. According to the preceding consideration, the first nuclear division was reductional and the second equational, and the diploid number

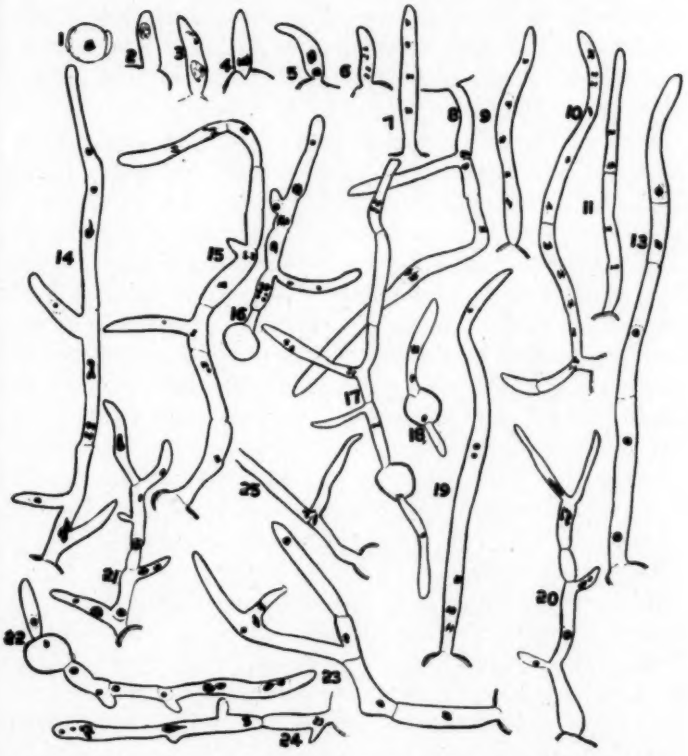


FIG. 1, *U. Williamsii.* 1, mature chlamydospore with a diploid nucleus; 2 and 3, young promycelia with one and two nuclei, respectively, in resting stage or very young prophase stage; 4, metaphase I, with four chromosomes; 5, anaphase I, 6, early anaphase II showing reduced number of chromosomes; 7, late anaphase II; 8 and 25, promycelia in which it is possible to observe a branch borne between adjacent cells, the breaking of the cross wall, the nuclear migration and the beginning of the dicaryophase; 9, late anaphase II or early telophase II; 10, promycelium with several nuclei, some in anaphase stage, just before branching and with the first cross wall formed; 11, four nuclei with the unreduced number of four chromosomes in each; 13, promycelium with a resting nucleus in each cell; 14 and 15, promycelia and branches with nuclei in different division stages (in this illustration cells without nuclei are shown); 16 and 17, promycelia with nuclei in third and fourth division stages, in which it seems that reduction did not take place; 17, bipolar germination in which more than two chromosomes appear in each nucleus; 18, the same as in figure 17, but with reduced nuclei; it is possible to observe that when nuclear division takes place inside the spore, one remains inside longer than the other; 16 and 20–24, dark bodies in variable number which seem to be chromatic or nuclear substance.

of chromosomes in this species is four. The promycelium elongates and soon a third equational division occurs and the four resulting nuclei enter telephase or undergo a new equational division (FIG. 1: 9, 10).

Figure 1: 10 shows a large promycelium with many nuclei, several of them in anaphase, in which it is possible to observe thread connections. Most of these seem to represent the late anaphase or early telephase. At the base there are short branches in which it is possible to observe a division at anaphase and it seems probable that one nucleus will migrate to the branch. In the same figure the first wall is clearly presented, dividing the promycelium into two cells. As soon as these nuclear divisions are finished, cell division is completed. In figure 1: 13 is shown a 5 celled promycelium with one nucleus in early anaphase and the others apparently in resting stages, each having a very thin membrane and deep stained nucleolus. In the resting haploid nuclei, a nuclear reticulum was not observed, but always a nucleolus located in a clear zone surrounded by a thin membrane. This corroborates Wang's (18) observations in *U. Avenae, U. levis,* etc., who said that "the haploid nuclei take the stain always in the same form: "the nucleolus is strongly stained and located in the nuclear cavity which is hyaline." If this is true, it is possible to take this characteristic to distinguish diploid from haploid nuclei.

According to the foregoing considerations, and as shown in figure 1: 4 to 7 and 9, and 10, the first nuclear division is reductional and the second equational. But, on the other hand, there is evidence that reduction division may take place in the second, third, or later nuclear divisions. Figure 1: 11 shows a promycelium with four nuclei in metaphase of the third division in which it is easy to count four chromatic bodies. Figure 1: 16 and 17 represent more advanced stages, and it seems that here reduction division did not take place, because there are infection hyphae. Apparently the number of chromosomes would have been reduced in the hyphae (FIG. 17). Sometimes secondary hyphae bear unreduced nuclei (FIG. 1: 23). Consequently no reduction took place in the first, second or third nuclear division. In contrast to this, in other cases, reduction division was not simultaneous for all the chromosomes

in the same stage nor at the same time. In fact, figure 1: 8, 15, and 19, show nuclei with two and three and four chromosomes.

There were observed in the promycelia and the infection hyphae of individual germinating spores many deeply stained bodies, variable in size and irregularly distributed, either in groups or singly. These bodies take the stain in the same manner as the nuclei (FIG. 1: 16, 20, 21, 24). In many cases these bodies appear along with the typical nuclei, in others they occur instead of the typical nuclei. Wang (**18**), in her study on *U. Avenae, U. Hordei,* etc., describes the behavior of the cytomes and vacuomes. These bodies are of vacular nature and stain deeply with haematoxylin. The illustrations of such bodies shown in Wang's paper suggest, in some respects, the kind of bodies observed in *U. Williamsii.*

Hyphae formation. Sometimes in the early stages of the development of the promycelium, in which reductional or equational nuclear division takes place, or in later stages, small branches begin to emerge from the promycelial cells and one nucleus migrates into each branch. Occasionally nuclear migration occurs when the branch is longer. Figure 1: 14 and 15 show young branches produced by means of the elongation of a promycelial cell containing one nucleus. It seems that these nuclei are produced just before branches begin, or very near to that time (FIG. 1: 20, 23). There are other types of hyphal formation, one of which is by means of fusion between adjacent cells. When this occurs, the wall which divided the cells begins to disappear and there emerges a short tube which contains the protoplasm of both cells. When this tube elongates, it seems that nuclear movement in the direction of the new hypha begins (FIG. 1: 8, 25). This marks the beginning of the dicaryophase (FIG. 1: 17). When reduction division takes place in the primary hyphae (FIG. 1: 12), the nuclei of the secondary hyphae are apparently haploid for all chromosomes and the dicaryophase begins in the secondary hyphae. The nuclear conditions were observed in only a few secondary hyphae, but it seems that there are secondary hyphae with and without the chromosomes reduced. Figure 1: 23 shows a secondary hypha with unreduced nucleus.

Therefore, it seems that there are three different kinds of hyphae: one that originates in one cell and has one or two reduced nuclei;

one that originates from two adjacent cells and has two conjugated nuclei; and another which originates from a single cell and has nuclei which are not reduced for all chromosomes.

USTILAGO SPEGAZZINII var. AGRESTIS Fisch. & Hirsch.

Ustilago Spegazzinii var. *agrestis* is characterized by dark-brown spores; smooth or very finely papillose epispore with bipolar crest, which varies from the entire apical thickenings to conspicuous, deeply dentate crest, hyaline, subhyaline to concolorous with the spore. Spores germinate after 40 hours at 25° C. A single germ tube emerges, elongates, and soon becomes differentiated more or less into a 3–4 celled promycelium, developing long branches. Occasionally a sporidium is formed at the tip of one or more of these branches. Sporidia were observed on plain or dextrose agar (7), but never in the nutrient solution used in the present studies. They branch and rebranch to initiate a mycelium.

Nuclear behavior. Mature chlamydospores contain one diploid nucleus each. At the beginning of germination the nucleus migrates toward the young tube when it is very short (FIG. 2 B: 1, 2). Nuclear division was observed inside the spore in only a very few cases, and it was impossible to determine the nuclear conditions. Figure 2 B: 1 and 3 show young promycelia with nuclei in the early metaphase with, apparently, four chromosomes in each one. Soon the promycelia elongate and it is possible to observe different anaphase stages in progress (FIG. 2 B: 4, 6), each having 2 chromosomes in each group. It seems that second nuclear division follows immediately and sometimes a third division. At this moment a cell wall appears forming two promycelial cells and as soon as the nuclei enter telaphase, the formation of the other walls begins, dividing the promycelium into 3–5 cells (FIG. 2 B: 7–11). In the nuclear behavior just described the first division is reductional and the second equational. The resting stages apparently are not produced, or, if they are produced, they are so short that they are not readily observed. Reduction does not always take place in the first division, as shown by the fact that 2 groups of 3 chromosomes each have been seen in the second metaphase stage (FIG. 2 B: 12). The nuclei divide a third time, again forming two nuclei just before branching of the promycelium begins.

Two kinds of hyphae were produced. Both were binucleate but one originated from fused adjacent cells and the other from individual cells. In the first case the nuclei do not always migrate simultaneously, but one may remain longer than the other in the promycelium cell (FIG. 2 B: 11).

In the older promycelium (FIG. 2 B: 13) resting nuclear stages were observed. The deeply stained nucleolus was located in a hyaline cavity surrounded by a very thin membrane, such as in *U. Williamsii* and in the species studied by Wang (**18**).

USTILAGO HALOPHILA Speg.

Chlamydospores yellow or yellow-brown, globose, sub-globose to slightly irregular, 5–7 μ diam. or 4–6 × 7–8 μ diam.; epispore smooth. The spores germinate after 8–12 hours at about 25° C. The promycelium elongates rather rapidly and soon becomes septate. Sometimes fusions occur between the cells of the promycelium which then give rise to infection hyphae. On plain agar, or in dextrose malt agar, sporidia often arise from the promycelial cells, or from the branches of the promycelium (**10**). In the cultures used in these studies sporidia were observed infrequently.

Nuclear behavior. The mature chlamydospores are uninucleate and the single nuclei are diploid as in other species. The first nuclear division may take place in the spore or in the promycelium. Inside the spore either one or two nuclei were observed. Presence of two nuclei indicates a preliminary division within the spore. Neither stages of division nor chromosomic behavior inside the spores was observed. Figure 2 A: 1 and 3 show very young promycelial tubes in which the nuclei migrated without a preceding division inside the spore, and in contrast to this, figure 2 A: 2 shows a spore in which the nucleus divided inside the spore and one nucleus migrated to the young promycelium while the other remained inside near the promycelium. The nucleus divides and one daughter nucleus migrates to the apex, as shown in figure 2 A: 4; the promycelium elongates and sometimes resting nuclear stages are observed (FIG. 2 A: 5). The promycelium elongates and there follows a second division after which a wall appears between each pair of nuclei. These walls divide the promycelium into 3 or 4

cells (FIG. 2 A: 7, 14, and 17). It was not possible to determine when reductional or equational nuclear divisions occur. In figure 2 A: 4, which represents a late telephase stage, it seems that each group contains two chromosomes, but in contrast to this, figure 2 A: 17 shows a very long promycelium containing nuclei with what appears to be more than two chromosomes. It seems more logical to consider that the last case (FIG. 17) represents an unreduced nucleus, and that before sporidial formation the nucleus undergoes another division, which would seem to be reductional. Although the promycelial cells usually fused and formed hyphae, some promycelia were observed to produce sporidia without fused cells.

Hyphae formation. There are two kinds of hyphal formation: One kind originates from adjacent fused cells and the other from fused alternate cells, the fusion in the latter case being accomplished by means of tubes. In the first case, when branching starts, a swollen point "knee joint" begins to form. At this point it is possible to observe the cell walls rupture and push out to the new formation which is followed by nuclear migration (FIG. 2 A: 8, 9, 10, 19). Sometimes the nuclei migrate simultaneously and at other times separately (FIG. 2 A: 10, 15). The knee joint seems to originate by means of elongation of the promycelial membrane, which results in the migration of the protoplasm of the involucrate cells, followed by the rupture and partial projection of the wall of the adjacent cell. Afterwards this membrane seems to split (FIG. 2 A: 8, 10,

FIG. 2 A, *U. halophila.* Figs. 1 and 3, germinating chlamydospores, each with a diploid nucleus in the young promycelium; 2, one nucleus in the promycelium and one in the spore, indicating that nuclear division took place in the spore; 4, first late anaphase or early telphase stage with reduced nuclei; 5, promycelium with nuclei in resting stage; 6, early and late anaphase stages; 7, mature promycelium with one nucleus in each cell; 8, 9, 10, 15 and 19, show young "knee joints" formed between adjacent cells; the formation of infection hyphae (the dicaryophase) has begun in several of them; also shown in some of the figures are simultaneous and separate migrations of the nuclei; 9, formation of infection hyphae between cells of the same promycelium by means of tube fusions; 11, 14 and 18, abnormal nuclear behavior or chromatic bodies of unknown sources; 14, the complete cross walls formed, in which is also shown abnormal chromatic bodies or abnormal nuclear behavior; 17, a four-celled promycelium, each nucleus in metaphase contains more than two chromosomes, or an unreduced number.

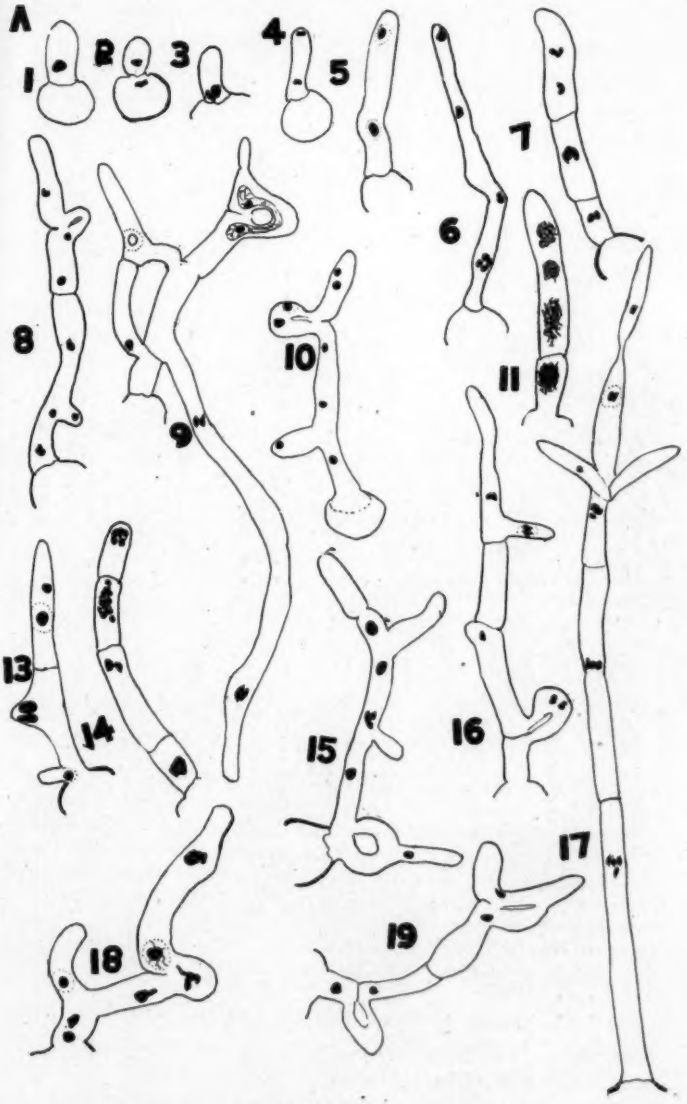


FIG. 2 A.

15, 16, 19). The "knee joints" represent the beginning of the dicaryophase and they soon develop large infection hyphae.

In the case of hyphae which originate by means of fusion of alternate cells, each cell produced a tube which soon becomes fused with a tube from another cell of the same promycelium, after which a

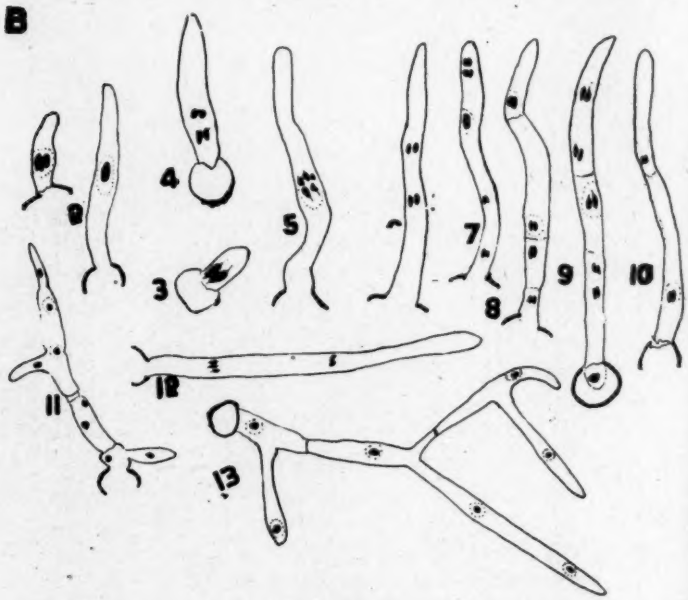


FIG. 2 B, *U. Spegazzinii* var. *agrestis*. Figs. 1 and 3, first metaphase stage in profile view; 2, resting nucleus; 4 and 6, early first anaphase, showing two chromosomes in each group; 5, early first anaphase stage with unequal distribution of chromosomes; 7–10, several telophase or late anaphase stages showing the beginning and completion of cell formation in the promycelium; 11, four promycelial cells with nuclei in the resting stage and young branches with nuclei in migration stages; 12, a promycelium with nuclei in second metaphase stage with different numbers of chromosomes; 13, promycelium branch with two conjugate nuclei and others in migrant stage.

large hypha containing two nuclei develops (FIG. 2 A: 9). In the same figure is shown a hypha containing two nuclei which originated from a cell without fusion.

Dark bodies of the type described for *U. Williamsii* were found in this species (FIG. 2 A: 11, 14, 18).

SOROSPORIUM CONSANGUINEUM Ellis & Ev.

Chlamydospores olivaceous, brown, irregularly polygonal, 7–10 μ diam.; epispore smooth. They begin to germinate after 9–12 hours in the incubator at 25° C. A tube emerges which elongates rapidly and in 3– 4 hours is completely developed, forming a 3–5 celled promycelium. Each promycelial cell bears one sporidium which sometimes forms secondary sporidia.

Nuclear behavior. Chlamydospores are uninucleate and diploid in the resting stage (FIG. 3: 1). When the spore germinates the young promycelium contains a deeply stained body which bears 4 or 5 chromatic elements; this appears to be the typical metaphase in profile and front view as shown in figure 3: 2, 3 and 4, respectively. Each one of these chromatic elements is prolonged by a thread so as to resemble a spindle. The promycelial tubes soon elongate and reduction division follows. Each group contains two chromosomes (FIG. 3: 5). The nuclei migrate toward the apex of the promycelium and at this stage the first promycelial wall is commonly produced (FIG. 3: 6). Apparently without a resting stage, these nuclei undergo a second division, such as that shown in figures 7 and 8. These illustrations represent what appears to be different anaphase stages. As soon as the nuclei are separated from each other, a wall forms between each pair and the promycelium becomes divided into cells (FIG. 3: 9, 10). These observations suggest that *S. consanguineum* possesses 4 chromosomes in the diploid stage and two in the haploid stage, and that the first division is reductional and the second equational. When division of the promycelium is complete, the nuclei undergo new equational divisions (FIG. 3: 10) just before sporidia are formed. One of the nuclei from each cell migrates into the sporidium and the other remains inside the promycelial cell. These divide again when new sporidia are formed.

The sporidia are soon separated from the promycelium and the nuclei divide again, sometimes two or three times, thus forming multinucleate sporidia (FIG. 3: 25). Worthy of note is one which seems to be near the last stage of nuclear division (FIG. 3: 25). Multinucleate sporidia are not common although Hanna (8) observed them in *S. Reilianum.*

Frequently spores of *S. consanguineum* germinated at low temperatures (8 to 10° C.), showed abnormalities in size in promycelium and nuclear behavior. These abnormalities were characterized by elongated promycelia, some of which were swollen cells, some with and some without cell formation. The nuclei of such promy-

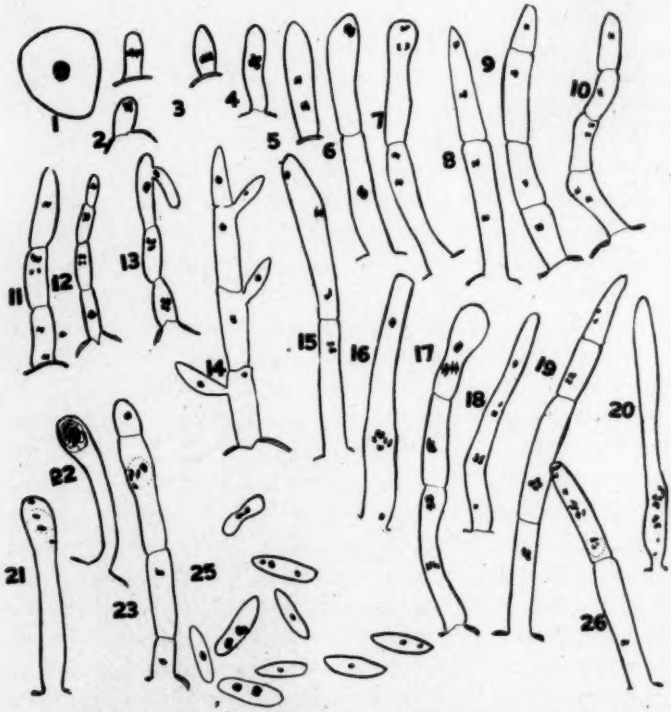


FIG. 3, *Sorosporium consanguineum*. 1, resting nucleus in mature chlamydospore; 2 and 3, first metaphase stage in very young promycelium with four chromosomes; 4, the same in front view; 5 and 6, early and late first anaphase stages with the number of chromosomes reduced to two in each group, first cross wall is shown in figure 6; 8, second anaphase stage; 9, late division stage showing mature promycelium; 10, equational division prior to sporidium formation; 11 and 13, nuclei in third division stage with a variable number of chromosomes in each group; 12, metaphase and anaphase stage, prior to sporidium formation; 14, sporidia developing from the promycelium following mitosis and the migration of one nucleus to each sporidium; 16–23 and 26, promycelia grown under low temperature showing abnormal development and nuclear behavior.

celia contained variable numbers of chromatic bodies. Figure 3: 11, 13, 16–23 and 26 illustrate these abnormalities.

DISCUSSION

It is known that segregation of factors for several characters occurs in the smuts either in the first, second, or later nuclear divisions in the promycelium (**5**, **6**, **11**, **16**, **17**, **18**). For the most part, the evidence that reduction occurs as late as the third division has been based on genetic studies, but Wang (**17**) has shown cytologically that reduction in *Ustilago Crameri* may occur also in the third division.

Evidence presented in this paper shows that reduction may occur in the third or later divisions in *U. Williamsii*, and that in *U. Spegazzinii*, var. *agrestis*, *U. halophila* and *Sorosporium consanguineum*, in the same promycelium there may be nuclei with variable numbers of chromosomes. However, in most cases the nuclei were reduced to 2 chromosomes before the formation of infection hypha or the sporidium. This was indicated by the fact that only a few remained with more than 2 chromosomes. Generally, therefore, the haploid number of chromosomes in these species is two. These apparently different types of chromosome reduction may be a source of different types of hyphae, and therefore of different biotypes.

As already pointed out, *U. Williamsii* produces three kinds of branches. One of these originates from a single promycelial cell and is haploid in nature. Consequently it cannot infect the host. Another kind originates from fused adjacent cells and has conjugate nuclei. This is a typical infection hypha. The third kind originates from a single promycelial cell and has only one nucleus which may be reduced for some characters but not for others. Therefore this kind may or may not be capable of infecting the host, depending upon whether reduction had occurred for sex. Thus, in its ability to produce this third kind of hypha *U. Williamsii* differs from other species which have been studied. Furthermore, it seems possible that hyphae of this type might represent "solopathogenic" lines, such as those found in *U. Zeae* (**2**).

In *U. Spegazzinii* var. *agrestis*, only two kinds of hyphae are produced, one of which develops from fused adjacent cells and the

other from individual cells. Both of these are binucleate but it could not be determined whether both kinds represent the dicaryophase. Infection hyphae, undoubtedly those which originate from single cells, are bisexual. On the other hand, those which originate from single cells may or may not represent the dicaryophase, depending upon whether the nucleus of the promycelial cell had been reduced for sex.

Hyphal formation in *U. halophila* is of two kinds, both of which are the product of fused promycelial cells. In one case the fusion is between adjacent cells by means of "knee joint" formations and in the other case fusion is between alternate cells by means of tubes. These differ, therefore, only in point of origin and both represent the dicaryophase.

Sporidial formation occurs occasionally in this species, in which case, fusion between sporidia or between mycelia produced by sporidia would precede the formation of infection hyphae. This process was not observed, however.

Sorosporium consanguineum differs from the other species studied, in that it produces sporidia instead of hyphae. Fusions between promycelial cells and between sporidia were not observed. The nuclear conditions of the sporidia while still attached to the promycelia indicated that they were haploid. In later stages the sporidia were largely multinucleate, probably as a result of nuclear divisions prior to budding. The absence of fusions probably was due to the fact that conditions were not favorable, since it seems logical that fusions may occur under natural conditions.

The significance of the abnormalities in the promycelium and in the nuclear behavior observed in *Sorosporium consanguineum* is not clear. It is known, however, that nuclear behavior in certain smut fungi is governed to some extent by environmental factors (**1**, **12**). In this case temperature seemed to exert a profound influence on type of germination and nuclear phenomena. The abnormal types usually appeared when the spores were germinated at a low temperature. In contrast, Wang (**17**) observed no influence of temperature on chromosome behavior in *U. Crameri*. This suggests, therefore, that different species may react differently to the same environmental factors.

The significance of the numerous darkly stained bodies of variable size and shape that were found in the promycelia of these species, along with nuclei that were in various stages of division, is not clear. They appeared to be chromatic in nature but the lack of uniformity in structure and behavior leaves one in doubt as to whether they actually are nuclear phenomena. Similar characters were referred to by D. T. Wang (**18**) as vacuomes and cytomes, which react to haematoxylin stain in the same manner as nuclei. On the other hand C. S. Wang (**17**) designated as nuclei, bodies of similar type in *U. Crameri*. Further studies with different staining and fixative methods will be necessary before definite conclusions can be drawn as to the nature of the bodies observed in the species studied. But in view of abnormalities due to unfavorable temperature, it may be suggested that unfavorable nutrient or other environmental factors were responsible for their appearance.

SUMMARY

1. Studies were conducted on the cytology of *Ustilago Williamsii, U. Spegazzinii* var. *agrestis, U. halophila,* and *Sorosporium consanguineum.*

2. The mature chlamydospores of *U. Williamsii* have a diploid nucleus. When the spore germinates the nucleus migrates to the promycelium where meiosis takes place. Apparently the diploid number of chromosomes is four. The reductional process may take place in the first, second, third or later nuclear divisions. In many cases primary and secondary hyphae possess unreduced nuclei. This is the first such case reported for smut fungi. In other cases it seemed there were hyphae in which reduction was complete for some chromosomes but not for others. Infection hyphae in this species develop by three means from the promycelial cells.

3. In *U. Spegazzinii* var. *agrestis* the mature chlamydospores contain one diploid nucleus. Except occasionally, when nuclear division apparently occurs inside the spore, the nucleus migrates to the promycelium where reduction division takes place. The diploid nuclei have four chromosomes. Two nuclei were observed in the late anaphase division stage. The first division on the promycelium is reductional but cases were observed in which it seemed that reduction did not take place in the first division. In this spe-

cies also, the promycelial cells developed into branched hyphae. These infection hyphae initiate the dicaryophase by means of nuclear migration from adjacent cells.

4. In *U. halophila* the mature chlamydospores are uninucleate and diploid. Nuclear division takes place in the promycelium, although in a few cases it was evident that division must have taken place inside the spore. The meiotic behavior was not clear, but in late stages of the second or third nuclear division it appeared that the haploid nuclei has 2 chromosomes. In some cases, nuclear reduction did not occur until sporidial or hyphal formation.

The infection hyphae may be originated by fusion between adjacent or alternate cells of the same promycelium. In the first case, this takes place by means of "knee joints" which seem to originate by elongation of promycelium membrane accompanied by the migration of the protoplasm and nuclei of the involucrate cells. This is followed by rupture and a partial projection of the membrane wall of the adjacent cells. The hyphae which originate as a result of fusion of alternate cells, do so when each cell elongates into a large tube which soon becomes fused with a tube from another cell of the same promycelium. After fusion is complete, a large hyphae develops, with nuclei from both cells. Uninucleate hyphae which formed from unfused cells also were observed.

5. In *Sorosporium consanguineum* the mature chlamydospore apparently possesses a diploid nucleus.

The meiotic process takes place in the promycelium. In the first division four chromosomes comprise the nucleus. In the second division it was possible to count only 2 in a group. Before sporidial formation the nucleus in the cell undergoes a new equational division and one nucleus migrates into the sporidium. The other remains inside the cell and sometimes divides again with the formation of additional sporidia. Sporidia with more than one nucleus were observed.

Low temperature seems to influence chromosome behavior during meiotic division resulting in unreduced nuclei and unequal distribution of chromosomes. Abnormalities in size and form of the promycelia were also produced.

The presence of many chromatic bodies of variable size and shape was observed in the four species studied.

LITERATURE CITED

1. **Bauch, R.** Multipolare sexualitat bei Brandpilzen. Zeits. Induk. Abs. Vererb. **54**: 258–259. 1930. According to Wang, C. S. 1943. (op. cit.)
2. **Christensen, J. J.** Studies on the genetics of *"Ustilago Zeae."* Phytopath. Zeitschr. **4**: 129–188. 1931.
3. **Dangeard, P. A.** Recherches sur la reproduction Sexuelle des champignons. Le Botaniste **3**: 221–281. 1893.
4. **Dasture, Jehangir Fardungi.** Cytology of *"Tilletia tritici"* (Bjerk.). Wint. Ann. Bot. **35**: 149. 1921.
5. **Dickinson, S.** Experiments on the physiology and genetics of the smut fungi. Hyphal fusions. Proc. Roy. Soc. London Ser. B. **101**: 126–136. 1927.
6. **Dickinson, S.** Experiments on the physiology and genetics of the smut fungi. Cultural characters. Par. 2. The effect of certain external conditions on their segregation. Proc. Roy. Soc. London Ser. B. **108**: 395–423. 1931.
7. **Fischer, G. W. & Hirschhorn, Elisa.** A critical study of some species of *Ustilago* causing stem smut on various grasses. Mycologia **37**: 236–266. 1945.
8. **Hanna, W. F.** Studies on the cytology and physiology of *Ustilago Zeae* and *Sorosporium Reilianum*. Phytopathology **19**: 415–422. 1929.
9. **Harper, R. A.** Nuclear phenomena in certain stages in the development of the smuts. Trans. Wisc. Acad. Sci. **12**: 475–498. 1898.
10. **Hirschhorn, Elisa & Fischer, G. W.** The smooth spored stem smuts of grasses of North and South America. Bull. Torrey Club (in press). 1945.
11. **Holton, C. S.** Further studies on oat smuts with special reference to hybridization. Cytology and Sexuality. Jour. Agr. Res. **62**: 229–240. 1941.
12. **Huting, W.** Ueber den Einflus der Temperatur auf die Keimung und geschlechtsverteiling bei Brandpilzen. Zeits. Bot. **24**: 529–557. 1931.
13. **Kharbush, S. S.** Contribution à l'etude des phenomenes sexuales chez les Ustilaginees. Ann. Sci. Nat. X. **9**: 285–297. 1927.
14. **Kharbush, S. S.** Recherches histologiques sur les Ustilaginees. Rev. Path. Veget et Ent. Afric. xv, 2 pp. 48056. *3 fig.* 1928.
15. **Rawlins, T. E.** Phytopathological and botanical research methods. New York. John Wiley and Sons Inc. London: Chapman and Hall. 1933.
16. **Stakman, E. C., Christensen, J. J., Eide, C. J., Peterson, Bjorn.** Mutation and hybridization in *Ustilago Zeae*. Minn. Agr. Exp. Sta. Tech. Bull. 65. 1929.
17. **Wang, C. S.** Studies on cytology of *Ustilago Crameri*. Phytopathology **33**: 1122–1133. with two plates. 1943.
18. **Wang, D. T.** Contribution à l'etude des Ustilaginees (Cytologie du parasite et la pathologie de la cellule hote). Le Botaniste **26**: 539–670. 1934. Extrait.

A CRITICAL STUDY OF SOME SPECIES OF USTILAGO CAUSING STEM SMUT ON VARIOUS GRASSES [1]

GEORGE W. FISCHER AND ELISA HIRSCHHORN [2]

(WITH 6 FIGURES)

INTRODUCTION

The name *Ustilago hypodytes* (Schlecht.) Fries has long been associated with stem smut on many grasses, and in many parts of the world. The original description was by Schlechtendahl (Fl. Berol. **2**: 129. 1824.) as *Caeoma hypodytes*. The type host is *Elymus arenarius* L., from Germany, but a large number of grasses have been reported as hosts since Fries recorded the species in the "Systema" (**9**).

During the course of a monographic study of the stem smuts of *Stipa* and *Oryzopsis* in North America (**8**), it was discovered that the binomial *Ustilago hypodytes* has for many years been applied to a complex of smut fungi, rather than to a single species. It is the purpose of this paper to present the results of a critical analysis of this complex, based on studies of hundreds of herbarium specimens from North America, South America, Europe, Asia, and Africa, most of which were identified as *U. hypodytes* by many prominent mycologists and issued as authentic exsiccati. Recent collections by the writers from North and South America are also included.

[1] Coöperative investigations of the smuts of forage grasses, by the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Washington State Agricultural Experiment Station, Pullman, Washington. Published with the approval of the director as Scientific Paper No. 599.

[2] Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and Assistant in Plant Pathology, State College of Washington, respectively.

The *Ustilago hypodytes* complex, as treated in this paper, embraces four species and one variety of smut fungi. These are separable into two groups: (1) Species whose spores possess bipolar areas or appendages; (2) Species whose spores lack bipolar areas or appendages.

I. STEM SMUT SPECIES WHOSE SPORES POSSESS BIPOLAR AREAS OR APPENDAGES

USTILAGO SPEGAZZINII Hirsch. Notas del Museo de la Plata, Botanica **4**: 415–419. 1939.

Ustilago hypodytes Auct.

Sori chiefly surrounding the internodes, but sometimes also involving more or less the inflorescence, although usually the inflorescence is entirely aborted, olive-brown to almost black, entirely naked except for the enveloping leaf sheaths; spores globose or sub-globose, to slightly oval or even somewhat angular, provided with bipolar sub-hyaline crests consisting of a prolongation of the epispore into a group of echinulations, finely papillose to minutely echinulate (oil immersion), clear yellowish-brown to olivaceous-brown, mostly 4–6 μ in diameter, or oval, 3.5–4 × 4–7 μ.

On GRAMINEAE:

Agropyron cristatum (L.) Gaertn. U. S.: Washington
Agropyron dasystachyum (Hook.) Scribn. U. S.: Washington
Agropyron inerme (Scribn. and Smith) Rydb. U. S.: Washington
Agropyron repens (L.) Beauv. U. S. Washington; Europe: Hungary, Spain, Germany, France; Africa: Tunis
Agropyron sibiricum (Willd.) Beauv. U. S.: Washington
Agropyron spicatum (Pursh) Scribn. U. S.: Washington
Agropyron trichophorum (Link) Richt. U. S.: Washington
Bromus erectus Hudson Europe: Germany
Elymus angustus Trin. ex Ledeb. U. S.: Washington
Elymus arenarius L. Europe: Germany
Elymus excelsus Turcz. U. S.: Washington
Elymus glaucus Buckl. U. S.: Washington
Melica Harfordii Boland. U. S.: Washington
Poa nevadensis Vasey U. S.: Washington

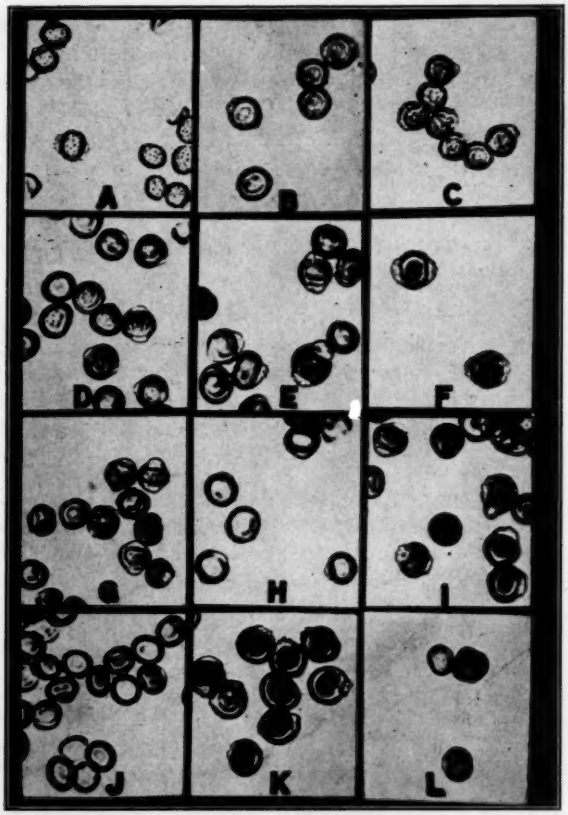


FIG. 1. A–C, *Ustilago Spegazzinii*, spores from: A, *Stipa Neesiana*, Argentin (loco typico); B, *Agropyron inerme*, United States; C, *Stipa Neesiana*, Bolivia; D–L, *Ustilago Spegazzinii* var. *agrestis*, spores from: D, *Agropyron rigidum*, Turkistan; E, *Lygeum spartum*, Sicily; F, *Stipa filiculmis*, Chile; G, *Agropyron acutum*, Italy; H, *A. repens*, Bohemia; I, *Bromus erectus*, Austria; J, *Elymus arenarius*, Sweden; K, *Lygeum spartum*, Spain; L, *Agropyron repens*, Germany (Sydow's *Ustilago agrestis*). All photomicrographs taken under same conditions of equipment, materials, lighting, and magnification. Somewhat retouched, to compensate where sharp focus is lacking. × approx. 800.

Poa palustris L. U. S.: Washington
Puccinellia distans (L.) Parl. U. S.: Washington
Sitanion hystrix (Nutt.) J. G. Sm. U. S.: California
Sitanion jubatum J. G. Sm. U. S.: Washington
Stipa mucronata HBK. (*S. setigera* Presl) Mexico
Stipa Neesiana Trin. and Rupr. South America: Bolivia, Argentina, Uruguay
Stipa robusta Scribn. (*S. vaseyi* Scribn.) U. S.: New Mexico
Stipa spartea Trin. U. S.: Illinois, Iowa, Wisconsin
Stipa viridula Trin. U. S.: N. Dakota, S. Dakota, Montana

During the course of these studies it has not been possible to refer to Schlechtendahl's original description of *Ustilago hypodytes*, nor has it been possible to obtain type material for study. Fries (**9**), in placing the species in the genus *Ustilago*, gives a description of macroscopic characters only. Thus it has been necessary to depend on mycological works subsequent to Fries' "Systema Mycologicum" for a description of the microscopic characters. In Table 1 is shown an analysis of the data given on the microscopic characters of *U. hypodytes* by twelve classical mycologists since Fries' time. The descriptions are strikingly similar. Of special importance is the fact that all these authors describe the epispore as *smooth*. Many of the specimens of *U. hypodytes* which we have studied are the same as those studied by some of the mycologists cited in Table 1. In the great majority of these specimens, on a wide variety of hosts, the spores have more or less the bipolar crests typical of *U. Spegazzinii*, either with or without the finely papillose epispore characteristic of the type material of the latter species. This observation applies to specimens representing the type host (*Elymus arenarius* L.) and the type locality (vicinity of Berlin, Germany) of *U. hypodytes*. Thus it is readily seen that (1) the presence of these characteristic crests has gone unrecognized or unheeded by numerous mycologists for several decades, and (2) apparently the name *Ustilago hypodytes* is a *nomen dubium*, under the provisions of Article 63, of the International Rules for Botanical Nomenclature. Only an examination of actual type material of *U. hypodytes* can positively verify these observations, but type material of this old species may no longer be available.

Three other species have been described since *Ustilago hypodytes*, as causing stem smut in various grasses and which have apparent similarities to *U. Spegazzinii*, both macroscopically and microscopically. These species are as follows:

Ustilago agrestis Syd. Ann. Myc. **22**: 278. 1924.
U. Bromi-erecti Cif. Ann. Myc. **29**: 51. 1931.
U. Stipae Cif. Ann. Myc. **29**: 52. 1931.

TABLE 1

TABULATION OF THE DATA GIVEN ON THE MICROSCOPIC CHARACTERS OF THE CHLAMYDOSPORES OF USTILAGO HYPODYTES BY VARIOUS MYCOLOGISTS

Author	Epispore	Dimensions	Color	Shape
Clinton (6)	smooth	4–7 μ in length		ovoid to spherical to irregular or polyhedral
McAlpine (14)	smooth	3–4 ×4–6 μ	dark olivaceous	globose to shortly ellipsoid
Migula (15)	smooth	3–4.5 ×3–6 μ	yellowish to olive brown	irregularly globose to slightly polyhedral
Ciferri (4)	smooth	4–5 or 4–5 ×3–6 μ	yellowish brown to olive brown	globose to ellipsoid
Liro (13)	smooth	3–5 ×4–6 μ	yellowish to brownish	more or less globose
Schellenberg (18)	smooth	3–5–7 μ	yellowish to brown	globose, ellipsoid or slightly angular
Saccardo (17)	smooth	3–4.5 ×3–6 μ	yellowish olive brown	globose, to ellipsoid, sometimes irregular to polyhedral
Plowright (16)	smooth	3–4.5 ×3–6 μ	yellowish brown	subglobose, oblong, or angular
Dietel (7)	smooth	4–5 μ	olive brown	globose
Winter (21)	smooth	3–5–6 μ	light brown	globose, irregularly globose, or polygonal
Lindau (12)	smooth	3–4.5 ×3–6 μ	yellowish olive brown	globose to irregularly ellipsoid
Bubak (3)	smooth	4–6, 7 μ	dark olive brown	globose to ovoid or angular

Although it has not been possible to examine type material of *Ustilago agrestis*, we have included subsequent material of this species identified by Sydow himself (Sydow, Mycotheca Germanica *2867*). Sydow (**20**) separated *U. agrestis* from *U. hypodytes* chiefly on the supposed specialization of the former to *Agropyron repens*. If Sydow observed the bipolar crests on the spores of his new species he made no mention of it in the species description, but the fact remains that the above-mentioned specimens have spores

which possess the bipolar crests and thus are very similar to other collections of stem smut on *A. repens* in the United States, Canada, Europe, and Africa.

Ciferri's (**4**) *Ustilago Bromi-erecti* is based on a biometric analysis of a collection of *U. hypodytes* on *Bromus erectus* Huds. from Austria. We have examined the same specimens (Zillig, Ustilag. Europ. No. 54) which Ciferri studied, and found the bipolar crests typical of *U. Spegazzinii.* However, Ciferri made no mention of these crests in his description of *U. Bromi-erecti.* The collection of stem smut on *B. erectus* on which Ciferri based his new species is very similar both macroscopically and microscopically to other collections of *U. hypodytes* on *B. erectus* from European countries. Both *U. agrestis* and *U. Bromi-erecti* have a smooth epispore.

Similarly, Ciferri (**4**) described *Ustilago Stipae* on a biometric basis, using two collections of *U. hypodytes* on *Stipa spartea* Trin. from the United States. We have included in our studies one of the two collections[3] forming the basis of Ciferri's "new" species. The spores of this collection have the bipolar crests and minutely echinulate epispore typical of *U. Spegazzinii.* Several other collections on *Stipa spartea* have the same characters. Ciferri made no mention in his description of *U. Stipae* of bipolar crests or of the minutely echinulate epispore.

From the above presentation it is seen that we have four binomials for this stem smut species which has bipolar crests on the spores, namely, *Ustilago agrestis, U. Bromi-erecti, U. Stipae,* and *U. Spegazzinii.* According to the descriptions, the first three species names have no connection with the principal diagnostic morphologic character, namely, the bipolar crests on their spores. It would appear, then, that these names are *nomina dubia.* The last name, *U. Spegazzinii,* is based on conspicuous morphological characters: bipolar crests on the spores and a minutely echinulate epispore.

Ustilago Spegazzinii was originally described (**10**) as occurring on *Stipa* only, and in Argentina. The results of the present studies show that many species of several other genera are also hosts, and in diverse parts of the world. On these hosts there is exhibited some variability in the morphology of the fungus. Thus it is pos-

[3] *U. hypodytes* on *Stipa spartea;* Meridian, Wisc., July 15, 1920, leg. J. J. Davis. Herb. Univ. Wisconsin.

sible to recognize, in the abundance of material examined, the following four groups:

Group I. Spores clear yellowish-brown, epispore minutely echinulate, crests comparatively inconspicuous, hyaline (FIG. 1, A).

Group II. Spores olivaceous-brown, epispore minutely echinulate, crests more or less inconspicuous, hyaline (FIG. 1, B and C).

Group III. Spores dark-brown, epispore finely papillose, crests more or less conspicuous, hyaline or concolorous (FIG. 1, D).

Group IV. Spores dark-brown; epispore *smooth*, crests variable, from inconspicuous bipolar entire thickened areas to conspicuous deeply dentate crests, hyaline (FIG. 1, E–L).

Of these four groups it is considered that the first two represent more or less typical *Ustilago Spegazzinii*. Group 3 differs from the typical *U. Spegazzinii* in having much darker spores, and with the epispore only slightly papillose. The fourth group is atypical with the smooth epispore, the variable crest, and dark color.

The third and fourth groups of *Ustilago Spegazzinii*, described above seem to comprise a distinct variety:

USTILAGO SPEGAZZINII var. **agrestis** (Syd.) comb. nov.

Spores dark-brown, smooth or very finely papillose (under oil immersion), crests varying from entire apical thickenings to conspicuous, deeply dentate crests, sub-hyaline to concolorous with the spore.

On GRAMINEAE:

Agropyron acutum (DC.) Roem. & Schult. Europe: Italy
Agropyron amurense Drobov U. S.: Washington (= *A. pendulinum* (Nevski) Swall.)
Agropyron cristatum (L.) Gaertn. U. S.: Washington
Agropyron elongatum (Host.) Beauv. U. S.: Washington
Agropyron glaucum Roem. & Schult. Europe: Switzerland (= *A. intermedium* (Host) Beauv.)

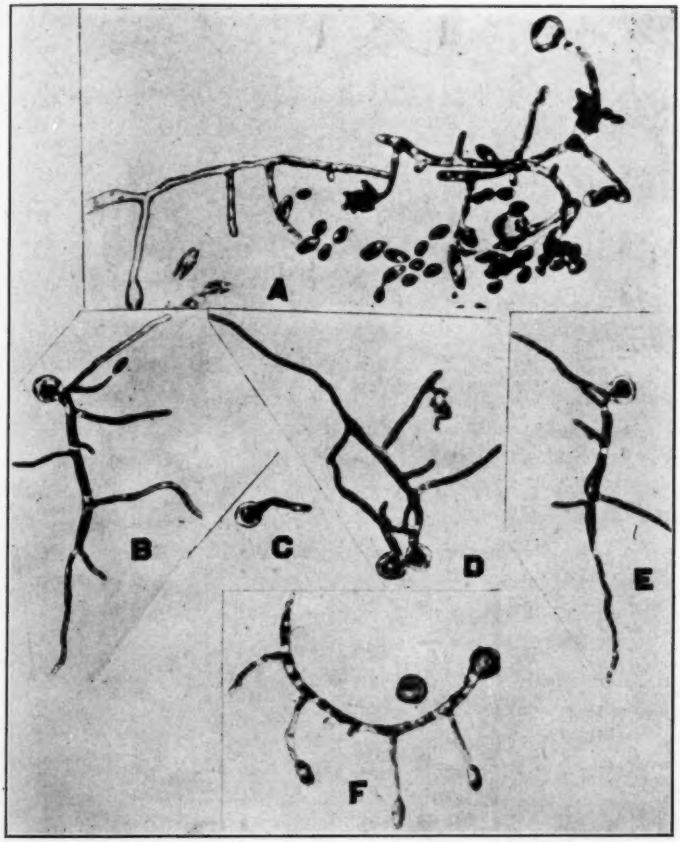


FIG. 2. A–E, germinating spores of *Ustilago Spegazzinii* var. *agrestis;* A, advanced germination, showing production of aerial sporidia, from *Agropyron Smithii,* Pullman, Wash.; B–E, ibid., from *A. intermedium,* Pullman, Wash., showing earlier stages of germination. Near spore in (E) a fusion has occurred from which an infection hypha is developing; F, germinating spore of *U. Spegazzinii,* showing production of sporidia, from *A. spicatum,* Goldendale, Wash. All spores germinated on malt extract-dextrose-peptone agar and stained *in situ* with cotton blue and lactophenol. All photographed under same conditions of light, apparatus, and magnification. Somewhat retouched, to compensate where sharp focus is lacking. × approx. 800.

Agropyron inerme (Scribn. & Smith) Rydb. U. S.: Washington
Agropyron intermedium (Host.) Beauv. Europe: Italy
Agropyron junceum (L.) Beauv. Europe: Great Britain
Agropyron Smithii Rydb. U. S.: Washington, Montana, Colorado
Agropyron spicatum (Pursh) Scribn. U. S.: Washington
Agropyron trachycaulum (Link) Richt. U. S.: Washington
Agropyron repens (L.) Beauv. U. S.: Washington, New York; Canada: Ontario, Sask., B. C.; Africa, Tunis; Europe: Spain
Agropyron rigidum (Schrad.) Beauv. Asia: Turkestan
Agropyron semicostatum (Steud.) Nees U. S.: Washington
Bromus erectus Huds. Europe: Germany, Switzerland, France
Elymus arenarius L. Europe: Germany, Scandinavia
Elymus canadensis L. . U. S.: Washington
Elymus condensatus Presl U. S.: Washington, Utah
Elymus dahuricus Turcz. U. S.: Washington
Elymus striatus L. U. S.: Montana
Lygeum spartum Loef. ex L. Europe: Spain, Italy; Africa: Algeria
Poa ampla Merr. U. S.: Washington
Poa scabrella (Thurb.) Benth. U. S.: Nevada
Sitanion Hansenii (Scribn.) J. S. Sm. U. S.: California
Sitanion hystrix (Nutt.) J. G. Sm. U. S.: California
Stipa californica Merr. & Davy U. S.: California, Mt. Shasta
Stipa filiculmis Delile South America: Chile
Stipa mucronata HBK. (*S. setigera* Presl) Mexico: Coronado Isl.
Stipa pulchra Hitchc. U. S.: California
Stipa spartea Trin. U. S.: Illinois
Stipa viridula Trin. U. S.: N. Dakota
Stipa sp. South America: Argentina

Group I is considered typical of *Ustilago Spegazzinii*. Group II is a color variation, with the spores being darker and less clear. These differences are relatively insignificant, and are merely recog-

nized as possibly indicating different forms only. The same applies to the differences between Groups III and IV. With a very few exceptions, all the collections studied have been readily classified in one of the four groups. For instance, one collection on *Stipa filiculmis* has the epispore and crest characters typical of *U. Spegazzinii*, but also the color of the variety *agrestis*. In another case, a collection on *Poa ampla* appears to be a mixture of *U. Spegazzinii* and the variety *agrestis*.

GEOGRAPHIC DISTRIBUTION

Ustilago Spegazzinii and the variety *agrestis* do not seem to be entirely co-existent. *U. Spegazzinii* has the following distribution, based upon the specimens we have examined: North and South America, central and southern Europe, and North Africa. The variety *agrestis* is represented in North and South America, Scandinavia, northern central and southern Europe, central Asia, North Africa. Furthermore, the variety appears to be more abundant than the species. In the United States, especially at Pullman, Washington, both the species and the variety (more especially the latter) are more or less common on several exotic grasses [4] including such species as *Agropyron amurense, A. cristatum, A. elongatum, A. junceum, A. rigidum, A. semicostatum, A. sibiricum, A. trichophorum, Elymus angustus,* and *E. dahuricus*. Of these, *A. cristatum* (crested wheat-grass) is commonly observed with 5–25 per cent infection in large fields planted for seed production, forage, or soil conservation. It is a matter of interesting conjecture as to the source of these infections. It seems possible that local perennial heavy infestations (25–95 per cent) on *A. repens* could represent one or more races capable of attacking these exotic species. Data are lacking concerning the actual importance of this stem smut elsewhere, except that in Argentina the junior author has observed as high as 25 per cent infection in *Stipa Neesiana* and *Stipa* sp.

[4] These grasses are planted in the extensive observational row nurseries of the Soil Conservation Service, Division of Nurseries, and the Forage Crops Nursery of the Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Dept. of Agriculture.

Other authors, Liro (13), Bornhövd (2), Ciferri (4), *et al.* cite many species in several other genera as hosts to *Ustilago hypodytes*. Doubtless many of these should be listed as hosts to *U. Spegazzinii* and the variety *agrestis*, but it is impossible to determine which, because at the present time specimens are not available supporting these citations.

Likewise, other mycologists list several synonyms under *Ustilago hypodytes*. It has not been possible to examine type material representing these binomials, but it is suspected that some of these may be associated with *U. Spegazzinii* and *U. Spegazzinii* var. *agrestis*.

SPECIMENS STUDIED

CANADA: On *Agropyron repens*, Saskatoon, Sask., 6–16–38, leg. and fid. R. C. Russel, Univ. Toronto Crypt. Herb. No. *638; ibid.*, Wilcox Lake, Ont., 6–14–36, leg. R. F. Cain, fid. H. S. Jackson, Univ. Toronto Crypt. Herb. No. *9553; ibid.*, Toronto, Ont., 6–8–36, leg. H. S. J. and R. F. Cain, fid. H. S. Jackson, Univ. Toronto Crypt. Herb. No. *9554; ibid.*, Toronto, Ont., 6–22–34, leg. H. S. Jackson, fid. H. S. J., Univ. Toronto Crypt. Herb. No. *6700; ibid.*, Westboro, Ont., 8–18–35, leg. M. Timonin, fid. ?, Univ. Toronto Crypt. Herb. No. *9827; ibid.*, Vineland, Ont., 6–9–39, leg. Richardson and McKeen, fid. H. S. Jackson, Univ. Toronto Crypt. Herb. No. *16122; ibid.*, Kleinburg, Ont., 9–8–35, leg. G. D. Darker, fid. H. S. Jackson, Univ. Toronto Crypt. Herb. No. *10200;* on *A. trachycaulum*, Summerland, B. C., 8–10–40, leg. M. F. Welch, fid. M. F. W. and H. S. Jackson.

UNITED STATES: On *Agropyron amurense*, Pullman, Wash. (S. C. S. Nursery),[5] 7–26–43, leg. E. Kreizinger, fid. G. W. Fischer, No. *329*, Myc. Coll. Bur. Pl. Ind. No. *85184;* on *A. cristatum*, Pullman, Wash., 6–21–37, leg. and fid. G. W. Fischer, No. *148*, Myc. Coll. Bur. Pl. Ind. No. *85004; ibid.*, Goldendale, Wash., 6–23–43, leg. and fid. G. W. Fischer, No. *322*, Myc. Coll. Bur. Pl. Ind. No. *85117; ibid.*, Pullman, Wash., 7–26–43, leg. and fid.

[5] Many of the collections from Pullman, Wash. have come from the forage nurseries at this station. Those from the Soil Conservation Service nurseries are indicated as "S.C.S. Nursery"; those from the Bureau of Plant Industry are indicated as "B.P.I. Nursery."

G. W. Fischer, No. *335,* Myc. Coll. Bur. Pl. Ind. No. *85190;* on *A. dasystachyum,* Pullman, Wash. (B. P. I. Nursery), June 1943, leg. and fid. G. W. Fischer; on *A. elongatum,* Pullman, Wash. (S. C. S. Nursery), 6–19–40, leg. and fid. G. W. Fischer, No. *149,* Myc. Coll. Bur. Pl. Ind. No. *85005;* on *A. inerme,* Pullman, Wash., 6–21–37, leg. and fid. G. W. Fischer, No. *145,* Myc. Coll. Bur. Pl. Ind. No. *85001; ibid.,* La Crosse, Wash., 6–21–43, leg. and fid. G. W. Fischer, No. *336,* Myc. Coll. Bur. Pl. Ind. No. *85191;* ibid., Prosser, Wash., 6–24–43, leg. and fid. G. W. Fischer, No. *330,* Myc. Coll. Bur. Pl. Ind. No. *85158;* on *A. intermedium,* Pullman, Wash. (B. P. I. Nursery), 7–26–43, leg. E. Kreizinger, and fid. G. W. Fischer, No. *332,* Myc. Coll. Bur. Pl. Ind. No. *85192;* on *A. repens,* Pullman, Wash., June 1937, leg. and fid. G. W. Fischer; *ibid.,* Colfax, Wash., July, 1940, leg. J. F. Schafer, fid. G. W. Fischer, No. *171,* Myc. Coll. Bur. Pl. Ind. No. *85027; ibid.,* Hall, N. Y., 7–9–23, leg. Martin and Haskell, fid. V. K. Charles, Myc. Coll. Bur. Pl. Ind.; on *A. semicostatum,* Pullman, Wash. (S. C. S. Nursery), June 1939, leg. and fid. G. W. Fischer, No. *166,* Myc. Coll. Bur. Pl. Ind. No. *85022;* on *A. sibiricum,* Pullman, Wash. (S. C. S. Nursery), 6–24–38, leg. and fid. G. W. Fischer, No. *168,* Myc. Coll. Bur. Pl. Ind. No. *85024;* on *A. Smithii,* Billings, Mont., July 1900, leg. Griffiths and Large, David Griffiths, West American Fungi, No. *234; ibid.,* La Jara, Colo., 8–14–1900, leg. and fid. C. L. Shear, No. *983,* Myc. Coll. Bur. Pl. Ind.; *ibid.,* Pullman, Wash. (B. P. I. Nursery), 6–24–38, leg. and fid. G. W. Fischer, No. *167,* Myc. Coll. Bur. Pl. Ind. No. *85023; ibid.,* 7–26–43, Pullman, Wash. (B. P. I. Nursery), leg. and fid. G. W. Fischer, No. *356,* Myc. Coll. Bur. Pl. Ind. No. *85210;* on *A. Smithii* var. *molle* Jones, Bozeman, Mont., 7–6–35, leg. H. A. Rodenhiser, fid. G. W. Fischer, No. *146,* Myc. Coll. Bur. Pl. Ind. *85002;* on *A. spicatum,* Pullman, Wash. (S. C. S. Nursery), 6–24–38, leg. and fid. G. W. Fischer, No. *165,* Myc. Coll. Bur. Pl. Ind. No. *85021; ibid.,* 7–2–43, leg. and fid. G. W. Fischer, Myc. Coll. Bur. Pl. Ind.; *ibid.,* Roosevelt, Wash., 6–23–43, leg. Fischer, Law, and Menzies, fid. G. W. Fischer, No. *339,* Myc. Coll. Bur. Pl. Ind. No. *85194;* on *A. trachycaulum,* Pullman, Wash., 7–2–36, leg. and fid. G. W. Fischer, No. *164,* Myc. Coll.

Bur. Pl. Ind. No. *85020; ibid.*, 7–24–43, leg. and fid. G. W. Fischer, No. *338*, Myc. Coll. Bur. Pl. Ind. No. *85193;* on *A. trichophorum*, Pullman, Wash. (B. P. I. Nursery), 7–3–40, leg. and fid. G. W. Fischer, No. *170*, Myc. Coll. Bur. Pl. Ind. No. *85026; ibid.*, Pullman, Wash. (S. C. S. Increase Plot), 7–2–43, leg. and fid. G. W. Fischer, No. *340*, Myc. Coll. Bur. Pl. Ind. No. *85195;* on *Elymus angustus*, Pullman, Wash. (S. C. S. Nursery), June 1939, leg. and fid. G. W. Fischer; on *E. canadensis*, Pullman, Wash. (S. C. S. Nursery), 7–16–40, leg. J. L. Schwendiman, fid. G. W. Fischer, No. *169*, Myc. Coll. Bur. Pl. Ind. No. *85025; ibid.*, 7–26–43, leg. and fid. G. W. Fischer, No. *332*, Myc. Coll. Bur. Pl. Ind. No. *85187;* on *E. condensatus*, Murray, Utah, Sept. 1918, leg. W. W. Jones, fid. A. O. Garret; *ibid.*, Dusty, Wash., 6–26–40, leg. and fid. G. W. Fischer, No. *150*, Myc. Coll. Bur. Pl. Ind. No. *85006;* on *E. dahuricus*, Pullman, Wash., 7–26–43, leg. E. Kreizinger, fid. G. W. Fischer, No. *331*, Myc. Coll. Bur. Pl. Ind. No. *85186;* on *E. glaucus*, Pullman, Wash. (S. C. S. Nursery), 6–24–38, leg. and fid. G. W. Fischer, No. 161, Myc. Coll. Bur. Pl. Ind. No. *85007;* on *E. striatus*, Billings, Mont., Aug. 1900, leg. Griffiths and Large, David Griffiths, West American Fungi, No. *201;* on *Melica Harfordii*, Pullman, Wash. (S. C. S. Nursery), June 1939, leg. and fid. G. W. Fischer, No. *163*, Myc. Coll. Bur. Pl. Ind. No. *85019;* on *Poa ampla*, Pullman, Wash. (S. C. S. Nursery), June, 1939, leg. and fid. G. W. Fischer, No. *162*, Myc. Coll. Bur. Pl. Ind. No. *85018; ibid.*, Pullman, Wash. (B. P. I. Nursery), 7–22–43, leg. and fid. G. W. Fischer, No. *342*, Myc. Coll. Bur. Pl. Ind. No. *85197;* on *P. scabrella* (Thurb.) Benth. (*P. buckleyana* Nash), Rabbit Hole, Nev., Aug. 1902, leg. and fid. D. Griffiths, No. *176*, Myc. Coll. Bur. Pl. Ind.; on *P. nevadensis*, Pullman, Wash. (S. C. S. Nursery), June 1938, leg. and fid. G. W. Fischer, No. *160*, Myc. Coll. Bur. Pl. Ind. No. *85016; ibid.*, June 1939, leg. and fid. G. W. Fischer, No. *159*, Myc. Coll. Bur. Pl. Ind. No. *85015; ibid.*, 6–14–40, leg. and fid. G. W. Fischer, No. *158*, Myc. Coll. Bur. Pl. Ind. No. *85014;* on *P. palustris*, Pullman, Wash. (S. C. S. Nursery), June 1939, leg. and fid. G. W. Fischer, No. *157*, Myc. Coll. Bur. Pl. Ind. No. *85013; ibid.*, June 1940, leg. and fid. G. W. Fischer, No. *156*, Myc. Coll. Bur. Pl. Ind. No. *85012;* on

Puccinellia distans, Pullman, Wash. (S. C. S. Nursery), 6–19–40, leg. and fid. G. W. Fischer, No. *155,* Myc. Coll. Bur. Pl. Ind. No. *85011;* on *Sitanion Hansenii,* vic. Mt. Shasta, Calif., 7–21–39, leg. W. B. Cooke, fid. B. L. Zundel, Cooke, Mycobiota of North America, No. *58;* on *S. hystrix,* vic. Mt. Shasta, Calif., 9–17–39, leg. W. B. Cooke, fid. G. L. Zundel, Cooke, Mycobiota of North America, No. *62; ibid.,* 8–20–39, leg. W. B. Cooke, fid. G. L. Zundel, Cooke, Mycobiota of North America, No. *59;* on *S. jubatum,* Pullman, Wash. (S. C. S. Nursery), June 1939, leg. and fid. G. W. Fischer, No. *152,* Myc. Coll. Bur. Pl. Ind. No. *85008;* on *Stipa occidentalis* Thurb., vic. Mt. Shasta, Calif., 8–22–39, leg. W. B. Cooke, fid. G. L. Zundel, Cooke, Mycobiota of North America, No. *61;* on *S. spartea,* Scatterwood, S. Dak., July, 1896, leg. D. Griffiths, Ex. Herb. David Griffiths, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Urbana, Ill., 6–21–88, leg. M. B. Waiter, fid. G. P. Clinton, Seymour and Earle, Economic Fungi, Supp., No. *C71; ibid.,* Grinnell, Iowa, 6–9–05, leg. B. Fink, fid. W. W. Diehl, Myc. Coll. Bur. Pl. Ind.; *ibid.,* Highmore, S. Dak., leg. G. R. Ball, fid. E. E. Dicks, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Meridian, Wisc., 7–18–20, leg. J. J. Davis, ex Herb. Univ. Wisc. (No. *19696* Herb. James R. Weir); on *S. robusta* (*S. vaseyi*), Paton Mts., N. Mex., 8–18–03, leg. and fid. D. Griffiths, No. *251,* Herb. Brooklyn Bot. Gard.; on *S. viridula,* Aberdeen, S. Dak., July 1895, ex Herb. D. Griffiths, Myc. Coll. Bur. Pl. Ind.; *ibid.,* Billings, Mont., 7–12–00, leg. Griffiths and Lange, fid. D. Griffiths, No. *252,* Herb. Brooklyn Bot. Gard.; *ibid.,* Morrison, Colo., 6–10–05, leg. E. Bethel, "Reliquiae Bethelianae," Myc. Coll. Bur. Pl. Ind.; *ibid.,* Manfred, N. Dak., 8–2–22, leg. Gilman Shirley, Brenckles' Fungi Dakotensis, No. *524; ibid.,* Sprague Siding, Minn., 6–3–41, leg. R. Sprague, fid. G. W. Fischer, No. *186,* Myc. Coll. Bur. Pl. Ind. *85042.*

MEXICO: On *Stipa mucronata* (*S. setigera*), Coronado Isl., 7–8–15, Elam Bartholomew's Fungi Columbiani, No. *4794.*

ARGENTINA: On *Stipa Neesiana,* La Plata, 1939, leg. and fid. E. Hirschhorn; on *Stipa* sp., Neugen, 2–1940, leg. and fid. E. Hirschhorn, No. *709.*

BOLIVIA: On *Stipa Neesiana,* Coleapirue, Feb. 1940, leg. M. Gardenas, No. *26A,* fid. J. A. Stevenson, Myc. Coll. Bur. Pl. Ind.

CHILE: On *Stipa filiculmis,* Freire, Dec. 1928, leg. Bro. Claude Joseph, No. *5818,* fid. J. A. Stevenson and A. J. Watson, Myc. Coll. Bur. Pl. Ind.

URUGUAY: On *Stipa Neesiana,* 1943, leg. B. Rosengurtt, fid. E. Hirschhorn, No. *1093.*

TURKESTAN: On *Agropyron rigidum,* Samarkand, July 1910, leg. Serebrianikow, Tranzschel et Serbrianjkow Myco-theca Rossica, No. *203.*

SWEDEN: On *Elymus arenarius,* 7–30–87, leg. C. J. Johanson, fid. Eriksson, Fungi Parisitici Scandinavia, No. *251; ibid.,* Sept. 1890, Varberg. Fungi Scandinavia, No. *1641; ibid.,* Scania, Aug. 1896, leg. G. Lagerheim, Vestergren Micromycetes rariores selecti, No. *391.*

ENGLAND: On *Elymus arenarius,* 1891, leg. Plowright, issued as Sydow Ustilagineen, No. *10;* on *Agropyron junceum,* near Liverpool, 3–8–97, leg. P. Magnus, issued as Vestergren Micromycetes rariores selecti, No. *1595.*

GERMANY: On *Agropyron repens,* Thuringen Erfurt, 6–26–04, leg. H. Diedicke, issued as Sydow, Mycotheca Germanica, No. *217; ibid.,* Berlin, June, 1896, leg. P. Sydow, issued as Sydow, Ustilagineen, No. *106; ibid.,* pr. Islebiam (Sax. Bor.), June, 1875, leg. and fid. J. Kunze, issued as Johs. Kunze, Fungi selecti exsiccati, No. *15; ibid.,* Lunderaberg in Mähren, June 1924, leg. R. Picbauer, issued as F. Petrak, Flora Bohemiae et Moraviae exsiccata, No. *1988; ibid.,* Hessen-Nassau; Geisenheim a Rh., 7–6–22, leg. G. Lüstner, issued as Zillig, Ustilagineen Europas, No. *23; ibid.,* Reuhof, 1894, issued as Herbier Barbey-Boissier, No. *1832;* on *Elymus arenarius,* Pommern: bei Zingst, 14–8–12, leg. P. Sydow, Sydow, Mycotheca germanica, No. *1164; ibid.,* Berlin, 10–1893, leg. P. Sydow, Sydow Ustilagineen, No. *10; ibid.,* Hazelhorst, 23–8–12, leg. and fid. A. Ludwig, Herb. Dr. A. Ludwig; *ibid.,* VII, 1895, ex. Herb. P. Magnus, Flora Pomeranica, No. 253; *ibid.,* Pommern, Kreis Usedorn-Wollin: Stranddünen bei Misdroy, 3–9–25, leg. Dr. Martin Noak, issued as Zillig's Ustilagineen Europas, No. *24.*

AUSTRIA: On *Bromus erectus,* Haschberg bei Klosterneuburg, 7–1925, leg. Dr. K. Keissler, issued as Zillig's Ustilagineen Europas, No. *54.*

BOHEMIA: On *Agropyron repens,* Ziegelschänke ad Trebritz, 25–7–00, by Fr. Bubak, issued as Vestergren's Micromycetes rar. selecti, No. *334.*

HUNGARY: On *Agropyron repens,* Budapest, June 1883, issued as Linhart, Fungi hungarici, No. *104.*

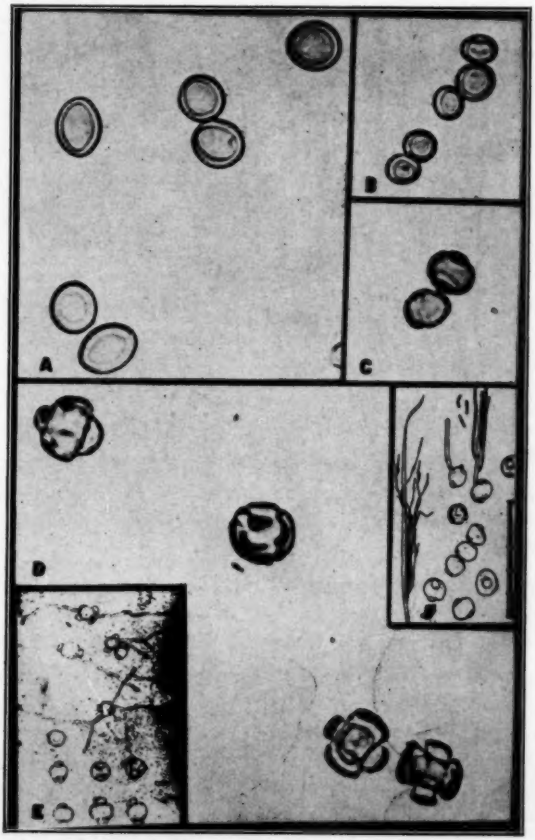


FIG. 3. A, *Ustilago halophila,* chlamydospores; type material; B, *U. nummularia,* chlamydospores, type material; C, *U. Williamsii,* chlamydospores, from Argentine specimen; D, *ibid.,* type material, from U. S.; E, Spegazzini's original illustrations of type material of *U. appendiculata* (*U. Williamsii*) after Hirschhorn; F, Lavrov's original illustrations of *Tranzschiella otophora* (*U. Williamsii*), after Zundel. A–D, × approx. 1000.

FRANCE: On *Agropyron repens,* Route de la Gare, Grignon, 3–6–35, Herb. G. Viennot-Bourgin, Flore cryptogamique de Seine-et-Oise: on *Bromus erectus,* Grignon, 20–VII–35, *ibid.*

SWITZERLAND: On *Agropyron glaucum,* Pris de Stalden, Vallu de St. Canton du Valais, July 26, 1914, leg. Dr. Eng. Mayor; on *A. intermedium,* Canton du Valais, 7–6–11, leg. E. Mayor, issued as Sydow, Ustilagineen, No. *452* and as Vestergren, Micromycetes rariores selecti, No. *1595 a;* on *Bromus erectus,* Canton de Neuchatel, 6–18–23, leg. Eug. Mayor.

ITALY: On *Agropyron acutum,* Rovigno, June 1902, leg. P. Sydow, issued as Sydow, Ustilagineen, No. *307;* on (?) *Lygeum spartium* (host not named), Cagliari, 1900, Briosi e Cavara Ustilaginee, No. *376;* on (? host not named), Briosi e Cavara Ustilaginee, No. *228.*

SPAIN: On *Lygeum spartum,* Casterlseras, leg. Loscos, issued as Rabenhorst, Fungi Europaei, No. *1800;* on *Agropyron repens,* Oredo, VII, 1919, Herb. del Museo Nacional de Ciencias Naturales de Madrid, No. *13081.*

SICILY: On *Lygeum spartum,* 1877, leg. V. Beltrani, issued as de Thuemen, Mycotheca Universalis, No. *930.*

ALGERIA: On *Lygeum spartum,* 4–18–06, leg. Rene Maire, issued as Vestergren, Micromycetes rariores selecti, No. *1205; ibid.,* Oran, 4–11–06, leg. R. Maire, issued as Sydow, Ustilagineen, No. *361.*

TUNIS: On *Agropyron repens,* Rossitz Morav., leg. Niessl, in Myc. Coll. Bur. Pl. Ind.

SPORE GERMINATION IN USTILAGO SPEGAZZINII AND U. SPEGAZZINII VAR. AGRESTIS

Hirschhorn (*10*) observed only 3 germinated spores in her studies of *Ustilago Spegazzinii* and provisionally described germination as follows:

"Las clamidosporas germinan en agua a 25° C. approximadamente, produciendo un promicelio 2–4 celular, con una esporidia en cada celulua."

On the basis of numerous observations of germinating spores of recent specimens (collected in United States) of *Ustilago Spegaz-*

zinii and *U. Spegazzinii* var. *agrestis* the process of spore germination may be briefly described as follows: On plain or nutrient agar, and at room temperature (20–22° C.), germination does not commence until after 40 hrs. or more. A single germ tube emerges (FIG. 2, C), elongates, and usually soon becomes differentiated more or less into a 3–4 celled promycelium. However, these cells have not been observed to produce primary sporidia, but instead long slender branches (FIG. 2, B, D, E). Occasionally a sporidium will be formed at the tip of one or more of these branches (FIG. 2, F) but more often the branches elongate and rebranch, to initiate a mycelium. Very soon aerial branches begin to bear chains of aerial sporidia (FIG. 2, A). Fusions have been noted between detached aerial sporidia.

USTILAGO WILLIAMSII (Griff.) Lavrov. Trav. Inst. Sci. Biol. Univ. Tomsk. **2**: 22. 1936.

Ustilago hypodytes Auct.

Sorosporium Williamsii Griff. Bull. Torrey Club **29**: 296. 1902.

Ustilago appendiculata Speg. Anal. Museo. Nac. De Buenos Aires III. **12**: 288. 1909.

Tranzschiella otophora Lavrov. Trav. Inst. Sci. Biol. Univ. Tomsk. **2**: 29. 1936.

Sori surrounding the upper internodes, often involving also remnants of the aborted inflorescence, dark-brown to black, naked except for the enclosing leaf sheaths; spores globose to sub-globose, provided with an exospore that is smooth but deeply cracked, and bearing two bipolar cap-like appendages (sometimes four), dark olivaceous-brown, 7–10 μ in diameter.

On GRAMINEAE:

Oryzopsis Bloomeri (Boland.) Ricker U. S.: Washington

Oryzopsis hymenoides (Roem. and Shult. Ricker. U. S.: Montana, Wyoming

Stipa californica Merr. and Davy U. S.: California

Stipa cernua Stebbins and Love U. S.: California

Stipa comata Trin. and Rupr. U. S.: Oregon, Montana

Stipa coronata Thurb. U. S.: California

Stipa humilis Cav. (*S. chrysophylla* Desv.) South America: Argentina

Stipa Lettermani Vasey U. S.: Wyoming
Stipa occidentalis Thurb. U. S.: California, Oregon
Stipa Richardsoni Link U. S.: Montana, Wyoming

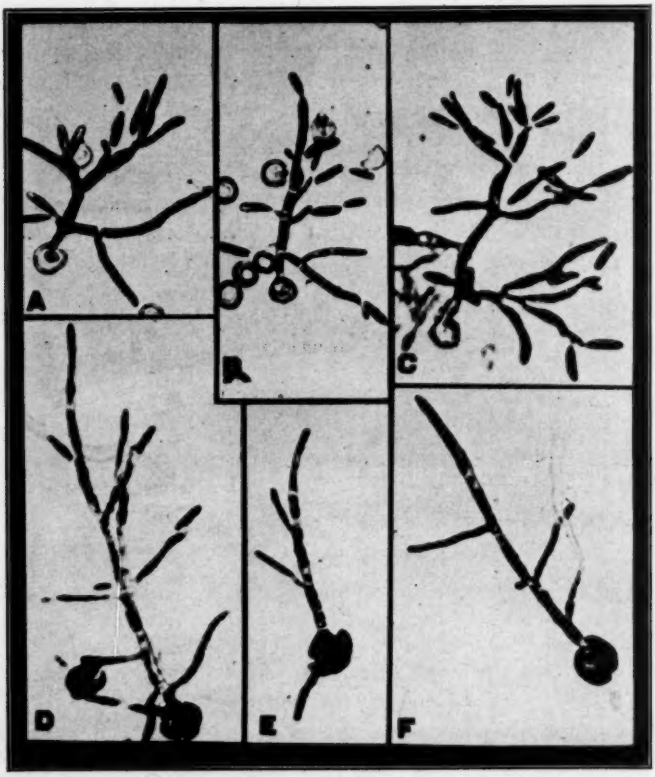


FIG. 4. A–C, *Ustilago halophila*, germinating spores, showing sporidial production; D–F, *U. Williamsii*, germinating spores. Germinated on malt extract-dextrose agar, and stained *in situ* with cotton blue in lacto-phenol. × approx. 800.

Stipa speciosa Trin. and Rupr. U. S.: California
Stipa Thurberiana Piper. U. S.: Washington, Oregon
Stipa viridula Trin. U. S.: Montana
Stipa sp. U. S.: California; South America: Argentina

Of the above list of host species, all except *Stipa humilis* (*S. chrysophylla*) and *S. Richardsoni* are new hosts.

A comparison of type material of *Ustilago appendiculata* (in Herb. Instituto de Botanica Spegazzini No. *3004,* Argentina, Mendoza, Cacheuta, Feb. 1909) and *Sorosporium Williamsii* (David Griffiths, West American Fungi No. *306* on *Stipa Richardsonii,* Big Horn Mts., Wyo., Col. Williams and Griffiths, Aug. 1898) leaves no doubt as to their identity. However, we do not consider this species as a *Sorosporium,* thus concurring with Clinton's (**5**) opinion that the "spore balls" of Griffith's species "result from a mechanical adherence of the spores in irregular masses." Since Griffiths' specific epithet has several years priority over that of Spegazzini we are proposing the new combination shown above. It is regrettable that the specific name *"appendiculata"* can not be conserved, because it is descriptive of the chief diagnostic character of this smut.

Recently Lavrov, according to Zundel (**22**), described a new genus of the Ustilaginaceae, *Tranzschiella,* the type species of which is *T. otophora* Lavrov, on *Stipa* in Siberia. According to the descriptions and illustrations of this species which Zundel (**22**) reproduced, it appears to be very similar to *U. Williamsii* and we are considering the two as synonymous, provisionally, until examination of type material makes a permanent disposition possible. Reproductions of Lavrov's illustrations of *T. otophora* and of Spegazzini's illustration of *U. appendiculata* are presented with our own of *Ustilago Williamsii* (FIG. 3, C, D, E, F) to show the apparent identity. The size and color of the spores, the appendaged or cracked character of the epispore, and the characters of germination are very strikingly similar. Hirschhorn (**11**) considered that *U. appendiculata* did not merit generic distinction because (1) the characters of spore germination are quite typical of *Ustilago;* (2) the sori are composed of a free, powdery or granular mass, as in most species of *Ustilago,* and (3) the presence of appendages on the spores is not sufficient in itself. The appendages of the spores of this smut are a character of the epispore. If such a character is accepted as a valid foundation for a new genus, then also a reticulate epispore could form the basis for a new genus, or such a

species as *U. Spegazzinii* with its characteristic bipolar prolongations of the epispore would equally merit generic distinction.

There are some variations in the character of the epispore of *Ustilago Williamsii* on different hosts in the U. S. that deserve mention. On some species of *Stipa* the spores are clear and yellowish, while on other species the spores are dark brown and opaque. In some specimens the epispore is deeply cracked to form the outlines of the appendages but these are largely lacking. In other collections portions of the cracked epispore have separated sufficiently to form conspicuous appendages.

SPECIMENS EXAMINED: ARGENTINA: Type material, *U. appendiculata* on *Stipa humilis* (*S. chrysophylla*), Herb. Instituto de Botanica Spegazzini No. *3004*. Mendoza, Cacheuta, Feb. 1909; on *Stipa* sp., leg. and fid. E. Hirschhorn, Mar. 1940, Hirschhorn Herb. No. *685*.

UNITED STATES: On *Oryzopsis Bloomeri*, Pullman, Wash. (S. C. S. Nursery), June 1940, leg. and fid. G. W. Fischer, No. *180*, Myc. Coll. Bur. Pl. Ind. No. *85036*; on *O. hymenoides*, Drummond, Mont., July 17, 1941, leg. Fischer and J. R. Hardison, fid. G. W. Fischer, No. *194*, Myc. Coll. Bur. Pl. Ind. No. *85050*; *ibid.*, Pinedale, Wyo., 7–23–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *204*, Myc. Coll. Bur. Pl. Ind. No. *85060*; on *Stipa californica*, Mt. Shasta, Calif., Cooke, Mycobiota of North America, No. *60*, fid. G. L. Zundel, 9–17–38; on *S. cernua*, Davis, Calif., leg. Stebbins and Love, fid. G. W. Fischer, No. *198*, Myc. Coll. Bur. Pl. Ind. No. *85054*; on *S. comata*, Big Timber, Mont., 7–19–41, leg. Fischer and J. R. Hardison, fid. G. W. Fischer, No. *195*, Myc. Coll. Bur. Pl. Ind. *85051*; *ibid.*, Billings, Mont., 7–19–41, leg. Fischer and J. R. Hardison, fid. G. W. Fischer, No. *197*, Myc. Coll. Bur. Pl. Ind. No. *85053*; *ibid.*, Demi, Ore., 8–1–01, Col. D. Griffiths (no. 249), Herb. Brooklyn Bot. Gard.; on *S. coronata*, Monrovia, Calif., leg. and fid. E. Bethel, Reliq. Bethelianae in Myc. Coll. Bur. Pl. Ind.; on *S. Lettermani*, Moran, Wyo., 7–22–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *200*, Myc. Coll. Bur. Pl. Ind. No. *85056*; *ibid.*, Triangle F Ranch, Wyo., 7–23–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *202*, Myc. Coll. Bur. Pl. Ind. No.

85058; on *S. occidentalis,* Ingo Nat. Forest, Calif., 8–5–25, leg. W. W. Wagener, fid. W. W. Diehl, Myc. Coll. Bur. Pl. Ind.; *ibid.,* Steins Mts., Ore., 1901, Griffiths, W. Amer. Fungi; on *S. Richardsoni,* Yellowstone Pk., Wyo., 7–21–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *199,* Myc. Coll. Bur. Pl. Ind. No. *85055; ibid.,* Butte, Mont., 7–18–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *203,* Myc. Coll. Bur. Pl. Ind. No. *85059; ibid.,* Big Horn Mts., Wyo., 1898, Griffith's West Amer. Fungi, No. *306* (type material of *Sorosporium* (*Ustilago*) *Williamsii*); on *S. speciosa,* Palmdale, Calif., 5–24–41, leg. H. W. Johnson, fid. G. W. Fischer, No. *212,* Myc. Coll. Bur. Pl. Ind. No. *85068; ibid.,* Banning, Calif., Griffith's W. Amer. Fungi, No. *250,* 5–14–06, in Herb. Brooklyn Bot. Gard.; *ibid.,* Mono Lake, Calif., 7–30–22, Reliq. Bethelianae, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Coll. Griffiths and Hunter, Griffiths Coll. No. *47* in Herb. Brooklyn Bot. Garden; on *S. Thurberiana,* Prosser, Wash., 6–24–43, leg. Fischer, Law, and Menzies, fid. G. W. Fischer, No. *348,* Myc. Coll. Bur. Pl. Ind. No. *85202; ibid.,* Pullman, Wash. (in forage plots), June, 1940, leg. and fid. G. W. Fischer, No. *181,* Myc. Coll. Bur. Pl. Ind. No. *85037; ibid.,* Mabton, Wash., 6–21–43, leg. Fischer, Law, and Menzies, fid. G. W. Fischer, No. *193,* Myc. Coll. Bur. Pl. Ind. No. *85049; ibid.,* between Goldendale and Toppenish, Wash., 6–23–43, leg. Menzies, Law, and Fischer, fid. G. W. Fischer, No. *347,* Myc. Coll. Bur. Pl. Ind. No. *85201;* on *Stipa viridula,* McLeod, Mont., 7–19–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *205,* Myc. Coll. Bur. Pl. Ind. No. *85061;* on *Stipa* sp., Pigeon Pass, Box Springs Mts., Calif., 6–3–31, leg. W. T. Horne, fid. H. B. Humphrey, A. G. Johnson, and W. W. Diehl, Myc. Coll. Bur. Pl. Ind.; on *Stipa* sp., Caliente, Calif., D. Griffiths Coll. No. 410, 7–22–04, Herb. Brooklyn Bot. Gard.; on *Stipa* sp., Laguna Mts., Calif., 7–20–20, Rel. Bethelianae, in Myc. Coll. Bur. Pl. Ind.; on *Stipa* sp., Lebec, Calif., 1920, Reliq. Bethelianae, fid. R. W. Davidson, in Myc. Coll. Bur. Pl. Ind.

SPORE GERMINATION IN USTILAGO WILLIAMSII

In our experience, the spores of *Ustilago Williamsii* germinate readily, on a variety of media. On malt extract-dextrose-peptone

agar, germination begins in four or five hours at room temperature. A rather slender promycelium emerges from one of the appendaged areas, and soon develops two or three cross-walls. From each of the cells thus formed a primary sporidium or a branch develops, as in figure 4, D and F. When only two cross-walls are formed three cells result in the promycelium, and the spore itself acts as the fourth cell, in which case a branch or sporidium usually emerges from the appendaged area opposite the promycelium (FIG. 4, E).

II. STEM SMUT SPECIES WHOSE SPORES LACK BIPOLAR AREAS OR APPENDAGES

In this category are treated two species of *Ustilago* in the *U. hypodytes* complex, whose spores are smooth and completely lack any bipolar areas or appendages.[6] Until now these species have been identified as *U. hypodytes*. We consider them to belong to two species not hitherto recognized as occurring in the United States.

USTILAGO HALOPHILA Speg. Anal. Museo. Nac. Buenos Aires III. 1: 58. 1902.

Ustilago hypodytes Auct.

Cintractia Distichlidis McAlp. Smuts of Australia, p. 169, Melbourne, 1910.

Sori surrounding the internodes and the rachis of the aborted inflorescence, enclosed at first by a thin grayish membrane (the host epidermis),[7] dark-brown to black, granular to pulverulent. Spores globose, ovoid, to slightly irregular, yellow or yellowish-brown to olivaceous-brown, smooth, chiefly 5–7 μ in diameter, or 4–6 × 7–8 μ (FIG. 3, A).

[6] *Ustilago minima* Arth. on *Stipa* and *Oryzopsis* has small smooth spores and superficially resembles the *U. hypodytes* complex. However, the sori are covered by a distinct and usually persistent membrane, and the species is therefore considered wholly unrelated to the other species herein treated.

[7] Spegazzini in his original description (7) makes no mention of a membrane around the sorus, but the type material plainly possesses it. We have found the membrane in all of the specimens of "*Ustilago hypodytes*" on *Distichlis* spp. examined. For this reason we have included mention of the membrane in the description of *U. halophila* above.

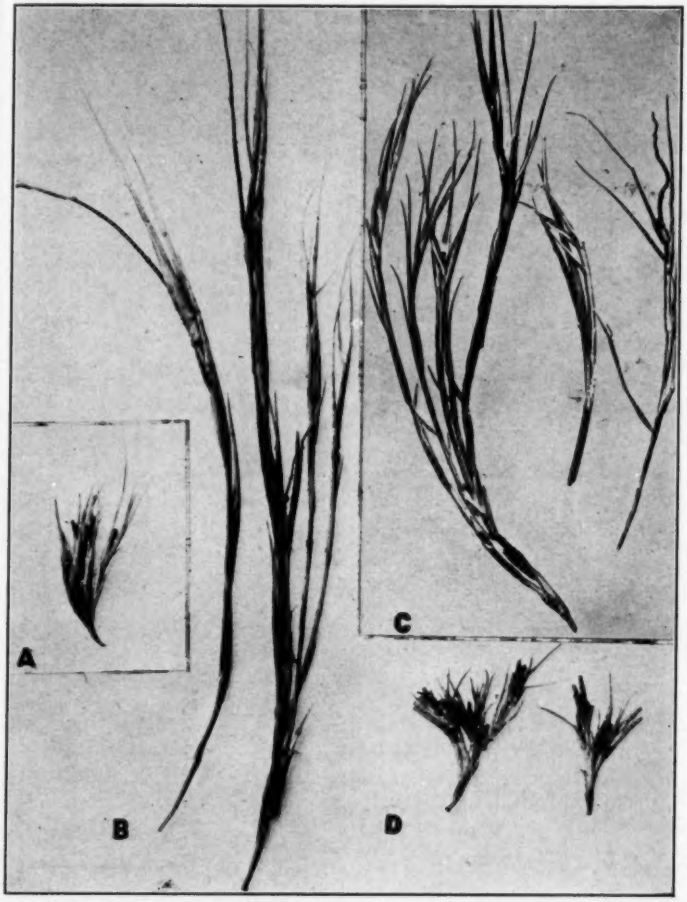


FIG. 5. *Ustilago halophila*. A, type material, on *Distichlis spicata* (Herb. Inst. Speg. No. 3198), illustrating the dwarfing effect; B, on *D. stricta*, without dwarfing effect, Salt Lake City, Utah, Fischer Coll. No. 173; C, McAlpine's *Cintractia Distichlidis* (*U. halophila*), to show similarity to elongate, American specimens; D, *U. halophila* on *Distichlis* sp. with dwarfing effect, Roswell, N. Mexico, Herb. D. Griffiths. Somewhat reduced.

On GRAMINEAE:

Distichlis spp.[8] U. S.: Idaho, New Mexico, Nevada, California, Washington, Arizona, Utah; South America, Argentina; Australia

The stem smut on *Distichlis* spp. common in the western states has been considered, until now, as *Ustilago hypodytes*. In Argentina, Spegazzini (**19**) described in 1902 a stem smut of *Distichlis spicata* (L.) Greene as *U. halophila*. We have studied type material of this species and are convinced that the stem smut of *Distichlis* in the United States is identical with it.

McAlpine (**14**) gave the name *Cintractia Distichlidis* to a stem smut attacking *Distichlis spicata* in Australia. We have not had the opportunity of examining type material of McAlpine's species, but his adequate description and excellent illustrations leave no doubt as to its identity with Spegazzini's *U. halophila* and with *"U. hypodytes"* on *Distichlis* in the United States.

Among the specimens examined during the present studies we have noticed three types of smutted plants of *Distichlis* spp. One is quite dwarfed (FIG. 5, A and D) and another is considerably taller (FIG. 5, B). A third type is intermediate between the two extremes. On the dwarf type the sori seem to be more or less confined to the last internode and the rachis of the aborted inflorescence. In the taller type the sori occupy several internodes. The spore characters are the same in both types and it is our opinion that the smut species is the same in both. Spegazzini's *Ustilago halophila* represents the "dwarfed" type (FIG. 5, A) and McAlpine's *Cintractia Distichlidis* apparently represents the taller type (FIG. 5, C) although he mentions that the host is sometimes much reduced in size due to the fungus (**6**). We are inclined to give no more significance to these three types than that they may represent three types of host species, not three types of smut. Clinton (**2**) states that *Distichlis maritima* Raf. (a synonym for *D. spicata* is sometimes very much dwarfed by stem smut.

[8] The hosts are given here as *Distichlis* spp. because it is our opinion that among the specimens we have examined there are some instances where the host species has been incorrectly determined; this also in view of Beetle's (**1**) recent demonstration of variation in the common species, *D. spicata*.

SPORE GERMINATION IN USTILAGO HALOPHILA

Spore germination in *Ustilago halophila* has been observed in recent collections from Washington and Utah. On dextrose malt extract agar, after 18–24 hours at room temperature (21–22° C.)

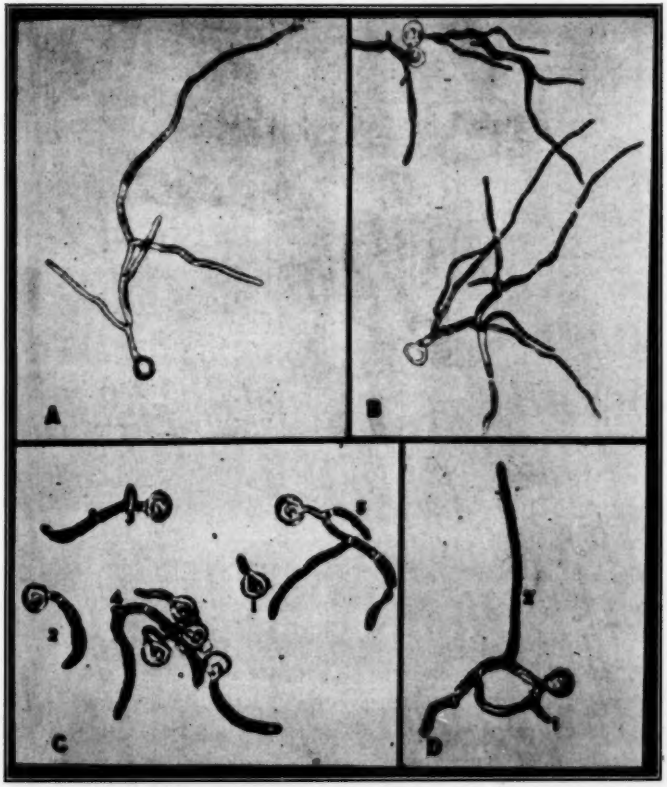


FIG. 6. A, B, *Ustilago nummularia*, germinating spores; C, *U. halophila*, germinating spores, fusions between adjacent cells are seen at 3 and 4, at 5 is a fusion tube from cell no. 1 about to contact and fuse with cell no. 3; D. *ibid.*, two infection hyphae developing from same promycelium; at 2 the infection hypha appears to have developed from the point of fusion between adjacent cells, at 1 the infection hypha is developing from the copulation tube connecting two remote cells (nos. 1 and 4). Germinated on malt extract dextrose agar and stained *in situ* with cotton blue in lactophenol. × approx. 800.

a germ tube begins to emerge from the spore (FIG. 6, C, 1). This elongates rather rapidly and soon becomes septate (FIG. 6, C, 2). Sometimes fusions occur between the cells of the promycelium (FIG. 6, C, 3 and 4). These fusions quickly give rise to infection hyphae (FIG. 6, D, 1 and 2). More often sporidia are borne from the promycelial cells or from branches of the promycelium (FIG. 4, A–C). This account comprises the first published description of spore germination in *U. halophila*.

SPECIMENS EXAMINED

ARGENTINA: Santa Fe; Rufino, Mar. 1900, Herb. Inst. Speg. No. *3198* (Type material) on *Distichlis spicata*. U. S.: Washington; Grant Co., on *Distichlis stricta* (Torr.) Rydb. col. D. C. Smith and J. R. Swallen, July 1940, fid. G. W. Fischer, No. *174*, Myc. Coll. Bur. Pl. Ind. No. *85030;* Blue Lake, on *D. stricta* 9–10–40, leg. and fid. G. W. Fischer, No. *357*, Myc. Coll. Bur. Pl. Ind. *85211;* Utah: Salt Lake City, on *D. stricta*, 7–16–40, col. G. W. Fischer, No. *123*, Myc. Coll. Bur. Pl. Ind. No. *85029;* Salt Lake City, on *D. stricta*, 8–24–14, A. O. Garret Herb. No. *2272;* Arizona: Empire Ranch, on *D. spicata*, leg. D. Griffiths, No. *174*, Herb. Brooklyn Bot. Gard.; New Mexico: Roswell, on *D. spicata*, 9–5–03, Vesterg. Micromycetes, No. *898* (Griffiths No. *205* in Herb. Brooklyn Bot. Gard.). Nevada: Quinn River Crossing, on *D. spicata*, 6–19–01, Herb. D. Griffiths, No. *235;* California Monterey, on *D. spicata*, Feb. 1910, leg., M. S. Clemens; Idaho: Roberts, on *D. spicata*, Aug. 1917, leg. F. S. Walpert, ex. Herb. James R. Weir, No. *8990*.

USTILAGO NUMMULARIA Speg. Anal. Museo. Buenos Aires III. 1: 59. 1902.

Ustilago hypodytes Auct.

Sori surrounding the internodes and sometimes extending into the aborted inflorescence, enclosed at first by the leaf sheaths, dusty, dark brown to almost black; spores globose to sub-globose, yellowish, brown, or olivaceous, smooth, chiefly 4–5 μ in diameter, or 3–4 × 4–5 μ.

On GRAMINEAE:

Ammophila arenaria (L.) Link Europe: Latvia

Ammophila arundinacea Host (Synonym of *A. arenaria*) Europe: Germany

Oryzopsis hymenoides (Roem. and Schult.) Ricker United States: Washington, Idaho, Utah, Colorado, Montana, Wyoming

Stipa comata Trin. & Rupr. United States: California, Oregon, Washington, Wyoming, Montana

Stipa neomexicana Thurb. & Scribn. United States: New Mexico

Stipa speciosa Trin. & Rupr. South America: Argentina

Stipa sp. United States: California

Of the above list of host species, all but *Stipa speciosa* must be considered as new host species to *Ustilago nummularia.*

The stem smuts of *Stipa* and *Oryzopsis* in North America having naked sori and small (4–7 μ), smooth spores without bipolar areas correspond to the various descriptions of *Ustilago hypodytes.* However, since we can not be certain as to the exact nature of the species to which this binomial was first given it becomes necessary to consider other names. Spegazzini in 1902 (**7**) described *U. nummularia* on *Stipa speciosa* in Argentina. An examination of type material of this species has revealed the identity of the North American stem smuts in question to Spegazzini's species.

SPORE GERMINATION IN USTILAGO NUMMULARIA

Germination has not been observed to begin in less than 2 days after sowing the spores on malt extract dextrose agar and incubation at room temperature (18–19° C.). A slender, hyaline germ tube emerges which develops rather rapidly, with sparse branching. Often the promycelium becomes 4-celled and from each cell a branch protrudes as though primary sporidia were in the process of development in the classic fashion of sporidia-producing smuts, but these protuberances merely develop into branches, as seen in figure 6, A. These continue to elongate and in turn branch (FIG. 6, B) and this process gives rise to a rapidly growing mycelium. Neither aerial nor surface-born sporidia have been observed.

SPECIMENS EXAMINED

UNITED STATES: on *Oryzopsis hymenoides,* Provo, Utah, leg. and fid. A. O. Garrett, No. *3129a; ibid.,* Near Edwin Natural Bridge, San Juan Co., Utah, 8–3–11, leg. and fid. A. O. Garrett, No. *2224; ibid.,* Emery Co., Utah, leg. and fid. A. O. Garrett; *ibid.,* San Juan Co., Utah, 7–22–11, leg. and fid. A. O. Garrett, No. *2178; ibid.,* Orondo, Wash., 8–11–26, leg. Zundel and Zentner, fid. G. L. Zundel; *ibid.,* Wolcott, Colo., 7–27–98, leg. D. S. Shear, fid. R. W. Davidson, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Glenwood Springs, Colo., 7–26–98, leg. Shear and Bessey, fid. R. W. Davidson, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Connel, Wash., 6–28–22, leg. and fid. George L. Zundel, Ustillaginales of the United States, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Orondo, Wash., 8–11–26, leg. G. L. Zundel and F. H. Zientner, fid. G. L. Zundel; Ustilaginales of the United States in Myc. Coll. Bur. Pl. Ind.; *ibid.,* St. Anthony, Idaho, Aug. 1900, leg. E. D. Merrill, fid. R. W. Davidson, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Glenwood Spa, Colo., 7–29–98, leg. C. L. Shear, fid. R. W. Davidson, in Myc. Coll. Bur. Pl. Ind.; *ibid,* Flockert's Ranch, Wyo., 6–30–01, leg. E. D. Merrill and E. N. Wilcox, No. *1197;* fid. J. C. Arthur, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Lind, Wash. (branch Exp. Sta.), 7–20–37, leg. and fid. G. W. Fischer, No. *176,* in Myc. Coll. Bur. Pl. Ind. No. *85032; ibid.,* Shelley, Idaho, Aug. 1937, leg. D. C. Smith, fid. G. W. Fischer, No. *177,* in Myc. Coll. Bur. Pl. Ind. No. *85033; ibid.,* Pinedale, Wyo., 7–23–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *188,* in Myc. Coll. Bur. Pl. Ind. No. *85044; ibid.,* Pullman, Wash. (S. C. S. Nurs.), 7–10–41, leg. and fid. G. W. Fischer, No. *196,* Myc. Coll. Bur. Pl. Ind. No. *85052;* on *Stipa comata,* Northville, S. Dak., 7–18–29, leg. and fid. J. F. Brenckle; Fungi Dakotenses, No. *675; ibid.,* Lakeside, Calif., 7–18–13, leg. E. Bethel, Reliquiae Bethelianae, in Myc. Coll. Bur. Pl. Ind.; *ibid.,* Woodward, Okla., 8–31–40, leg. C. L. Lefebvre, fid. G. W. Fischer, Myc. Coll. Bur. Pl. Ind. No. *85043; ibid.,* Moro, Ore., 7–17–35, leg. Virgil Hawk, fid. G. W. Fischer, No. *174,* Myc. Coll. Bur. Pl. Ind. No. *85031; ibid.,* Cody, Wyo., 7–20–41, leg. Fischer and Hardison, fid. G. W. Fischer, No. *192,* in Myc. Coll. Bur. Pl. Ind. No. *85048; ibid.,*

Pasco, Wash., 6–24–43, leg. A. G. Law and G. W. Fischer, fid. G. W. Fischer, No. *354,* Myc. Coll. Bur. Pl. Ind. No. *85208;* on *Stipa neomexicana,* Santa Fe, New Mexico, 8–23–26, leg. Bros. G. Arsene and A. Benedict, fid. G. P. Clinton and G. L. Zundel, in Myc. Coll. Bur. Pl. Ind.; on *Stipa* sp. (no host given on packet), Nevada City, Calif. July 1926, leg. L. S. Smith, fid. G. L. Zundel; Ustilaginales of the U. S., in Myc. Coll. Bur. Pl. Ind.

CANADA: On *Oryzopsis hymenoides,* Beaver Creek, Sask., 8–28–38, leg. T. Stevenson, fid. R. C. Russel, No. *711* in Univ. of Toronto Crypt. Herb.

ARGENTINA: On *Stipa speciosa* (Type material), La Plata, 1900, leg. Spegazzini, in Herb. Inst. Speg. d. Bot. No. *3619.*

LATVIA: On *Ammophila arenaria,* Düna, 16–VII–1903, leg. F. Bucholtz, ex Herb. Inst. Phytopath. Univ. Tartuensis.

GERMANY: On *Ammophila arundinacea* (synonym of *A. arenaria*), Berlin, 1876, leg. P. Sydow, issued as de Thuemen, Mycotheca Universalis, No. *820.*

LITERATURE CITED

1. **Beetle, Alan A.** The North American variations of *Distichlis spicata.* Bull. Torrey Club **70**: 638–650. 1943.
2. **Bornhövd, L.** Beiträge zur Biologie von *Ustilago hypodites* (Schldl.) Fr. auf *Elymus arenarius* L. Phytopath. Zeitschr. **9**: 69–97. 1936.
3. **Bubak, F.** Die Pilze Böhmens. II Teil, Branapilze (Hemibasidii). 81 pp. Prague. 1916.
4. **Ciferri, R.** Quinta contrbuzione allo studio degli Utilaginales. Ann. Myc. **29**: 1–74. 1931.
5. **Clinton, G. P.** North American Ustilagineae. Proc. Bost. Soc. Nat. Hist. **31**: 329–529. 1904.
6. ——. Ustilaginales. North American Flora **7**: 1–82. 1906.
7. **Dietel, P.** Hemibasidii, in Engler und Prantl, Die Naturlichen Pflanzenfamilien. I. (1): 2–24. 1900.
8. **Fischer, George W.** The stem smuts of *Stipa* and *Oryzopsis* in North America. Butler Univ. Botanical Studies **7**: 1945. (In press.)
9. **Fries, Elias.** Systema Mycologicum **3**: 518. 1832.
10. **Hirschhorn, Elisa.** Una nueva especie de *Ustilago* de la flora Argentina. Notas del Museo de La Plata, Botanica **4**: 415–419. 1939.
11. ——. Las especies del genero "*Ustilago,*" en la Argentina. Darwiniana **3**: 347–418. 1939.

12. **Lindau, G.** Ustilagineen, Auricularineen, Tremellineen, in Kryptogamenflora der Mark Brandenburg. Pilze III: 1–68. Gebrüder Borntraeger. Leipzig. 1914.
13. **Liro, J. I.** Die Ustilagineen Finnlands I. Ann. Acad. Scient. Fenn., Ser. A. **17**: 1–636. 1924.
14. **McAlpine, D.** The smuts of Australia. 288 pp. Melbourne. 1910.
15. **Migula, W.** Rost und Brandpilze. Handbucher für die Praktische Naturwissenschaftliche Arbeit. **13**: 1–132. 1917.
16. **Plowright, C.** A monograph of the British Uredineae and Ustilagineae. 347 pp. London. 1889.
17. **Saccardo, P. A.** Sylloge Fung. **7** (2): 453. Berlin. 1888.
18. **Schellenberg, H. C.** Die Brandpilze der Schweiz., *in* Beiträge zur Kryptogamenflora der Schweiz. **3** (2) 1–180. 1911.
19. **Spegazzini, C.** Mycetes Argentinensis. An. Mus. Nac. de Buenos Aires, III. **1**: 58–59. 1902.
20. **Sydow, H.** Notizen uber Ustilagineen. Ann. Myc. **22**: 277–291. 1924.
21. **Winter, G.** Ustilagineae, in Rabenhorst, Kryptogamen-flora von Deutschland, Oesterreich, und der Schweiz. Die Pilze I. Schizomyceten, Saccharomyceten, und Basidiomyceten. 79–132. Leipzig. 1884.
22. **Zundel, George L.** Notes on the Ustilaginales of the World. III. Mycologia **35**: 164–184. 1943.

PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XXXIX. A NEW HELOTIUM

FRED J. SEAVER

(WITH 1 FIGURE)

In November 1938, the writer received from Mr. Maurice Walters of Cleveland, Ohio, a rather unusual cup-fungus. In form it resembled a minute *Cudonia* but did not fit any of the plants described for this genus, either in consistency or in size, being much too minute.

It was finally decided, on more critical examination, that it was a stipitate *Helotium* in which the apothecium soon became convex and *Cudonia*-like. However, because of the uncertainty of its identity it was not published, although the illustrations had been prepared.

In October 1944, H. M. Fitzpatrick sent specimens collected by one of his students, Mr. Richard P. Korf, in Ithaca, which are identical with those sent from Cleveland. This has prompted me to publish the illustrations made several years ago with records and descriptions. Since the fungus resembles in form species of *Cudonia* it is described under the following name:

Helotium cudonioides sp. nov.

Apotheciis stipitatis, gregariis, vel caespitosis dein convexis 2 mm. diam., brunneis; stipitis albidis vel pallidis, 2–3 mm. long., vix 1 mm. diam.; ascis clavatis 8-sporis, 100 μ long., 10–12 μ diam.; sporiis subdistichis, fusoideis, hyalinis 5 × 16–20 μ; paraphysibus filiformibus, 1 mm. diam.

Apothecia stipitate, single or cespitose, at first concave, soon becoming strongly convex, reaching a diameter of 2 mm., pale to dark brown; stem white or nearly white, reaching a length of 2–3 mm. and less than 1 mm. in diameter; asci clavate, 8-spored reaching a length of 100 μ and a diameter of 10–12 μ; spores 1-seriate below and irregularly 2-seriate above, fusoid, hyaline, about 5 × 16–20 μ; paraphyses filiform, 1 mm. or less in diameter.

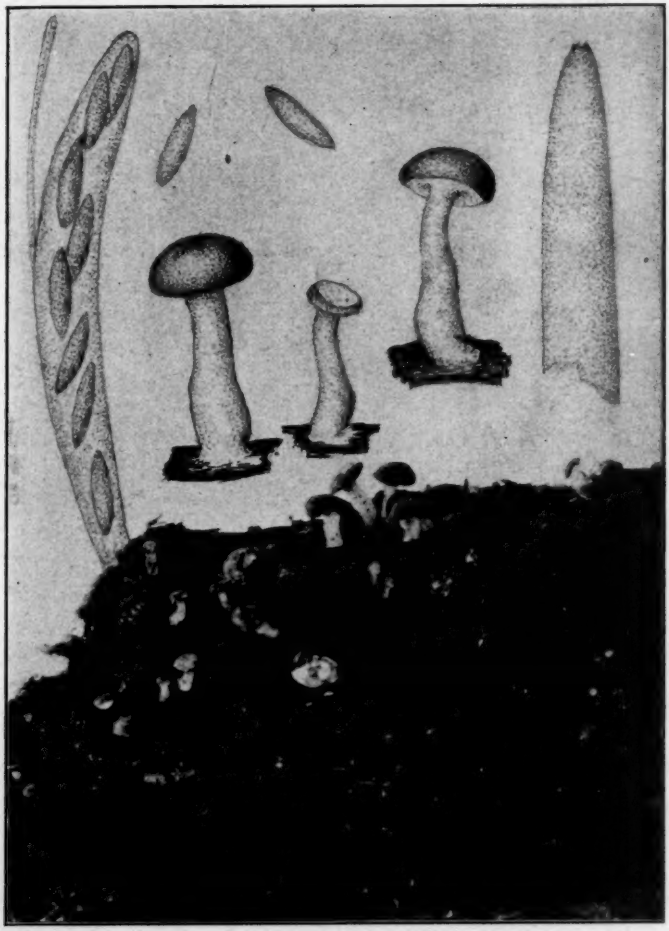


FIG. 1. *Helotium cudonioides.*

On rotten wood.

Type locality: Cleveland, Ohio.

Distribution: New York and Ohio.

NEW YORK BOTANICAL GARDEN

EXPLANATION OF FIGURE

Lower, photograph of a group of apothecia on rotten wood about 2½ times enlarged; above, drawings of three apothecia in different stages and from different angles; left, ascus with spores and paraphysis and two spores freed; right, empty ascus showing ascostome.

NOTES AND BRIEF ARTICLES

Apparent Allies of Amanita verna

The spores of *A. verna* are globose. Species that resemble it macroscopically are *A. bisporigera, A. hygroscopica, A. virosa, A. magnivelaris, A. gemmata volvata,* etc. In Florida a number of species in this genus have elongate spores, and these species curiously enough exhibit lines of evolution similar to those with globose or ellipsoid spores. The two here described, for example, might be mistaken for *A. verna* when fresh but their spores are elongate.

Venenarius margarita sp. nov.

Pileo convexo-plano, 5–6.5 cm. lato, albido, grato; lamellis liberis, confertis, albis; sporis oblongis, 10–11 × 3–4 μ; stipite albo, floccoso, 8 × 1–1.3 cm.; bulbo magno, albo; volva ampla, 2 cm. lata; annulo magno, albo.

Pileus convex to plane, scattered, 5–6.5 cm. broad; surface slightly viscid when wet, smooth, glabrous, dull-white with an avellaneous tint, pearly when dry, slightly yellowish on the disk, sometimes with one or two volval patches, margin even, entire; context very thin, white, unchanging, odorless, mild; lamellae just free, rounded behind, ventricose, inserted, close, medium broad, fimbriate, white, unchanging; spores amyloid, oblong, smooth, about 10–11 × 3–4 μ; stipe slightly tapering downward to bulb, white, unchanging, glabrous at the apex with faint lines running down from the gills, floccose below the ring, about 8 × 1–1.3 cm.; rooting base 2.5 × 1.5 cm., volva limb white, flaring, about 1.5 cm. high and 2 cm. broad; annulus large, white, persistent, fixed 1 cm. from apex.

Type collected by W. A. Murrill under a laurel oak in Gainesville, Fla., June 28, 1944 (*F 38906*). Remarkable for its pearly appearance when dry. Rare.

Venenarius tenuifolius sp. nov.

Pileo conico-expanso, gregario, 6–8 cm., subviscido, glabro; lamellis adnatis, confertis, albis, fimbriatis; sporis cylindricis, 12 × 5 μ; stipite bulboso, albo, 7 × 0.7–1.3 cm.; volva vaginata; annulo parvo, albo.

Pileus conic to expanded, gregarious, 6–8 cm. broad; surface slightly viscid, smooth, glabrous, shining when dry, white, becoming yellowish on the disk with age or on drying, margin even, entire; context thin, fragile, white, unchanging, odorless; lamellae adnate, close, narrow, thin, much shriveled on drying, white, unchanging, fimbriate; spores cylindric, smooth, about $12 \times 5\ \mu$; stipe enlarged downward to the large bulb, white, unchanging, pruinose above, glabrous and shining below, about 7×0.7–1.3 cm., bulb 2.5 cm. thick; volva large, membranous, white, persistent, sheathing, with ragged margin; annulus small, white, fixed very near the apex of the stipe.

Type collected by W. A. Murrill in soil at the base of a laurel oak in Gainesville, Fla., June 22, 1944 (*F 38002*). Ten hymenophores were found growing in a very small area. They suggested *V. vernus* but the stems were too short. The cylindric spores, of course, distinguished them at once. In age or when drying there is a slight carrion odor. Rare.

For those using Saccardo the species described above are recombined as follows:

VENENARIUS MARGARITA = **Amanita margarita**
VENENARIUS TENUIFOLIUS = **Amanita tenuifolia**

W. A. MURRILL

PROF. V. A. TRANZSCHEL, 1868–1942

In the passing of Vladimir Andreevich (Woldemar Heinrich) Tranzschel on January 21, 1942 in besieged Leningrad science lost one of the oldest and most outstanding of Russian mycologists, a specialist of international standing on Uredineae.

Tranzschel was born on Jan. 4(16), 1868 in St. Petersburg and was educated at the University of the same city, where his teachers were such well known botanists as A. N. Beketov, C. Gobi, A. A. Famintzyn and I. P. Borodin. His strong inclination to botany urged him to join a small group of students, such as A. N. Krasnov, Robert von Regel, N. I. Kuznetsov, etc., who spent all their spare time in field botanical work and on heated discussion of taxonomic problems, and all of them were to become prominent scientists in the future. It was a good school for a young, energetic and hard

working student of natural history. He early became interested in cryptogams and his thesis work—*An enumeration of Uredineae of St. Petersburg Province* (Russian, 1888)—is an important contribution to that field of botany.

After his graduation in 1889 he became assistant in cryptogamic botany and curator of the Botanical Museum of the St. Petersburg University but the next year he went to St. Petersburg Institute of Forestry as an assistant of Prof. Borodin. Here he had an opportunity to study the rich mycological flora of the famous park of the Institute. In 1897 he worked for some time in the laboratory of the Biological Station at Bologoe and published a long enumeration of the fungi of the Valdai region (1901). From that time he started to pay more and more attention to biology and phylogeny of fungi. In 1898 he was transferred to the University of Warsaw as an assistant in plant morphology and systematics but in 1900 returned to St. Petersburg to become curator of the Botanical Museum of the Academy of Sciences. Since then, he was always connected with the Academy, becoming senior botanist of the Institute of Botany in 1912, the position he held until his death.

Tranzschel was an outstanding research worker, and a keen observer, who combined a perfect experimental technique with profound and cautious evaluation of experimental results.

His chief interest was in Uredineae to the study of which he devoted more than 50 years of his fruitful life. He was first to establish and prove experimentally the relationship between aecidial stages of rusts and their teleutospores on various host plants. It is known as "law or method of Tranzschel" and it is his most important contribution to the science of mycology. This problem is discussed in the two following works: *Ueber die Möglichkeit die Biologie wirtswechselnder Rostpilze auf Grund morphologischer Merkmale vorauszusehen* (1904) and more in detail in *La règle Fischer et la méthode Tranzschel" chez les Uredinées* (Russian, 1934).

Among his other works can be mentioned, as more important, *Contribution à l'étude du genre Triphragmium auct.* (1923) in which he divided this genus into three genera (*Triphragmium* Link, *Triphragmiopsis* Naum. and *Nyssopsora* Arth.) on the ground of

the affinity of host plants affected by these rusts. His interest in the phylogeny and evolution of fungi found expression in two important works—*Die Rostpilze in ihrer Beziehung zur Systematik der Gefässpflanzen* (1927) and *Les Uredinées comme indicateurs de l'affinité de leur hôtes en rapport avec l'évolution phylogénétique de ces champignons* (Russian, 1936). The results of his fifty years' study of rusts of Russia are incorporated in one of his last and most important works—*Rusts of the USSR* (Russian, 1939) in which he described 844 species of rusts occurring in the USSR and 288 species which possibly exist in that country. The parasites of each family are segregated into natural groups.

When Tranzschel came to the Botanical Museum of the Academy, the cryptogamic herbarium was practically non-existent. Now it may be considered one of the best in Europe. But it would be a great mistake to consider Tranzschel as a mere herbarium botanist. He was too active for such a role. He was an ardent traveller and collector. As a young man he collected in Vyborg (Vipuri), Novgorod and St. Petersburg provinces. In 1899 he visited Germany, Austria and Switzerland and in 1903 again was in Germany and in Switzerland, where he studied the mycological flora of the Swiss Alps. His trips in Russia were so numerous that we can mention only the most important ones. He explored Kirghizistan in 1900 and collected in Alai and Transalai Mts. (Turkestan). He made two trips to Ussuri and Primorsk regions in 1927 and 1929, the result of which he presented in two papers—*Uredinalium species novae ex Sibiria* (1933) *and Zur Biologie der Uredineen des Fernen Ostens* (1940). He also collected for a number of years in the Crimea.

He always identified his own collections as well as those of his numerous correspondents. He also cultivated an exchange of specimens with European and American mycologists. All this material of great scientific value is deposited mostly in the Cryptogamic Herbarium of the Institute of Botany at Leningrad.

Besides this, he took a very active part in editing with A. A. Jaczewski and V. L. Komarov, *Fungi Rossiae exsiccati*, 7 fasc. (1895–1900), and started in 1910 in collaboration with V. A. Serebriannikov another publication of a similar kind under the title *Mycotheca rossica*, 7 fasc., which existed until 1912.

Although of ripe age, Tranzschel was very active; besides his routine work, he lectured in many educational institutions, was a prolific writer and always took part in all scientific meetings and conferences. The strain of terrible sufferings in besieged Leningrad broke his health and added the name of this distinguished scientist, whose work was not only of great theoretical value but of immense practical importance to his native country, to the long list of victims of total war.

The sources consulted: A. S. Bondartsev, *The seventieth anniversary of birth of Prof. V. A. Tranzschel,* etc. in Priroda (1938) (4): 147–153, and R. Singer in Science **99**: 443. 1944.—VLADIMIR C. ASMOUS.